ISSN : 2347 - 9213

Indian Journal of Current Research (IJCR)

(Quarterly International Journal)

Vol.2, February 2015

Special Issue No.1

**CHEMISTRY AND EFFICACY OF NATURAL PRODUCTS-
A DEMAND IN GREATER USE OF PLANT BASE DRUGS**

BON SECOURS COLLEGE FOR WOMEN
(Affiliated to Bharathidasan University, Tiruchirappalli)
(UGC Recognized 2(f) and 12(B) Institution)
VILAR BYPASS, THANJAVUR - 613 006, TAMIL NADU
Phone: +91-4362-257447, 255939, Fax: +91-4362-255939
Website: www.bonsecourscollege.org
Email id: bonsecourscollege02@gmail.com

JOURNAL COMMITTEE

Patron	:	**Rev. Sr. Jeyarani FBS** Secretary
Chief Editor	:	**Dr. Sr. D. Victoria Amalorpava Mary FBS** Principal
Associate Editors	:	**Ms. S. Muthu Bharathi** Head, Dept of Chemistry
		Mrs. A. Amargeetha Assistant Professor Dept. of Chemistry
		Mr. T. Joseph Pandian IQAC Co-Ordinator

Editor–in Chief, Editorial Board and Publisher take no responsibility for inaccurate, misleading data, opinion and statements appeared in the articles and advertisements published in the journal. It is the sole responsibility of the contributors and advertisers. No part of this journal can be reproduced without the written permission of the Editor- in- Chief who also holds the copyright © of the "Indian journal of current research (IJCR)".

Editorial...

Phytomedicine or the use of herbal medicine with therapeutic properties has played a significant role throughout history. Although its usage greatly diminished during the dawn of the scientific era, there is a revival of interest in its potential by late 20th century, especially in the development of new drugs. The history of herbal medicine can be traced back to thousands of years in both Western and Eastern tradition. Herbal medicine can be categorized into phytotherapy, over-the-counter herbal and traditional herbalism..

The role of phyto medicine started to decline after the 1960s as vast quantities of resources and money were used to promote synthetic medication. Besides this, advances in the human genome, increase knowledge of the structure and function of proteins and the notion that synthetic drugs are safer with fewer side effects also contributed to the rise in the popularity of synthetic drugs. However, these advancements have several major constraints. The large number of possible new drug targets has already outgrown the number of existing compounds that could potentially serve as drug candidates and the field of chemistry has limitation when it comes to synthesizing new drug structures.

In the last decade, herbal medicine has seen some form of revival, advancing at a greater pace in community acceptance of their therapeutics effects. This field is bringing forward new lead drug discoveries as well as safe and efficacious plant-based medicines. In turn, this leads to growing number of sales of commercialized medicinal herbs and most importantly, growing number of pharmaceutical companies that involve in the research and development of plants as a source for modern medicine. What chemists have been desperately seeking, Mother Nature has already plenty of stock. This edition tries to expound on the importance of plant base products in modern drug development by highlighting salient topics on chemistry and efficacy of phyto medicine and examining its roles in modern drug development. In addition, this review discusses the challenges and future of herbal medicine in modern medical practice.

Editors

CONTENTS

S. No.	Title	Page No.
1.	***In silico*** **Analysis of Bioactive Flavonoids as Potential Inhibitors of Indoleamine 2,3-Dioxygenase** *M.S. Abarna Priya, R. Gayathri Devi, B. Kameshwari, K. Ramaraj & Ronaldo Anuf. A**	1
2.	**Phytochemical Analysis and in Vitro Antioxidant Activities of Ethanolic Leaf Extract of *Mimosa Pudica*** *Deepa. R, Selvakumar .K, Saravanan.R & Krishnamoorthy .G*	5
3.	**Growth and Characterization of Tryptophan Doped with Paracetamol** *G. Madhurambal & B. Kavitha*	11
4.	**Molecular Docking Studies of Phytoconstituents from *Allium Sativum* against Glycogen Synthase Kinase 3β Protein** *Radhika.S, Ramaraj.K & Ronaldo Anuf.A**	18
5.	**Antidiabetic Effect of Ethanolic Root Extract of *Salacia Reticulata* in Alloxan Induced Diabetic Albino Rats** *J. Revathy*	21
6.	**Review on Nanoparticle - An Innovative Development in Cancer Therapy** *Sajel. S, Praveen. D & Ranadheer Chowdary.P*	28
7.	**Kinetics of Batch Adsorption of Iron(II) Ions From Aqueous Solution using Activated Carbon from Corchorus Olitorius-L-Leaves** *S. Subasri, S. Arivoli, V. Marimuthu & N. Mani*	33
8.	**Research Update on *Caesalpinia Bonduc* (L.): A Brief Review** *Subbulakshmi. P*	44
9.	**"GC-MS Analysis of Methanol Extracts of Flowers of *Allamanda Neriifolia Hook*"** *R. Sumathi*	49
10.	**Eco-Friendly Dyeing of Cotton Fabric using the Natural Mordants** *S. Thiyagarajan, K. Balakrishnan & S. Tamilarasi*	54

IN SILICO ANALYSIS OF BIOACTIVE FLAVONOIDS AS POTENTIAL INHIBITORS OF INDOLEAMINE 2,3-DIOXYGENASE

M.S. Abarna Priya, R. Gayathri Devi, B. Kameshwari, K. Ramaraj & Ronaldo Anuf. A*

Department of Biotechnology, Kamaraj College of Engineering and Technology, Virudhunagar, Tamilnadu

Corresponding Email id: ronaldoanuf@gmail.com

Abstract

Flavonoids are most abundant naturally occurring polyphenlic constituents present in variety of fruits, vegetables and various dietary supplements. They form the most essential link between diet and prevention of chronic disorders. Evidences suggest the potential of these flavonoids in prevention of various disorders such as inflammation, cardiovascular and neurological disorders. The aim of the present study was to explore the activity of selected bioactive flavonoids on immunosuppression enzyme Indoleamine 2,3-dioxygenase (IDO) for restoring tumor immunity. IDO is found highly expressed in diverse cancer types. Molecular Docking analysis was performed using Molegro docking software. Of the selected flavonoids the compound Naringin showed more promising results with a Mol Dock score of -171.73. The mode of interaction was also stronger.

Keywords: *Flavonoids, Molegro, Indoleamine 2, 3-dioxygenase*

Introduction

Bioactive compounds are extra nutritional constituents that typically occur in smaller quantities in foods. Flavonoids are a family of polyphenolic compounds which forms an integral part of human diet. Flavonoids are synthesized by plants and it is present in food sources like fruits, spices, seeds, stems, flower, vegetables, nuts, cereals as well as in tea, red wine etc.,(Holden J et al.,2002).Flavonoids are the key products which provide important link between diet and prevention of chronic disorders (Ramaraj K et al., 2014). It was validated through both invitro and invivo models that these bioactive compounds can inhibit tumor formation and proliferation of cancer cells through various biological mechanism of action (Vasilis P. et al., 2010). Flavonoids exert anticancer actions via a combination of mechanisms such as carcinogen inactivation, ant proliferation, cell cycle arrest and induction of apoptosis, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance (Gibellini et al., 2011: Chahar et al., 2012). Flavonoids are known to reduce the risk from several forms of human cancer like lung cancer, colon cancer, ovarian cancer and breast cancer.

Tumors have developed various strategies to escape immune attack (Zou et al., 2005). Recently, indoleamine 2,3-dioxygenase (IDO) a molecule capable of preventing T cell-driven rejection of allogeneic fetuses during pregnancies (Munn DH et al., 1998) has attracted the attention of scientists. Indoleamine 2,3-dioxygenase (IDO) is an intracellular heme enzyme that catalyses the initial and rate-limiting step in the metabolism of the essential amino acid tryptophan along the kynurenine pathway. (Soliman H et al., 2010). First, tryptophan depletion can directly lead to T-cell growth arrest in the G1 phase of the cell cycle. Second, alternative degradation of tryptophan produces metabolites shown to be toxic for CD8+ T cells and natural killer cells. Furthermore, IDO has the ability to convert naive T cells to immunosuppressive regulatory T cells (Fallarino F et al., 2006). High levels of IDO expression are found in patients with ovarian carcinoma, hepatocellular carcinoma, invasive cervical carcinoma, non-small cell lung carcinoma, colon carcinoma and endometrial carcinoma and are associated with poor prognosis (De Jong RA et al., 2012). Elevated tryptophan catabolism in the urine and blood of tumor-bearing patients has been recognized for many decades, perhaps explained by the discovery of common IDO1 overexpression in tumors. Immunohistochemical analysis of colon tumors indicated that IDO1 overexpression is associated with a significant reduction of CD3+ infiltrating T cells and higher occurrence of liver

metastases in colorectal cancer patients. In case of ovarian cancer IDO1 was found to be overexpressed in serous-type ovarian cancer where it was associated with decreased patient survival. IDO1 overexpression was also associated with resistance to paclitaxel. In the present study, we investigated the effect of selected bioactive flavonoids in suppressing the activity of the Indoleamine 2,3-dioxygenase protein. Furtherthe binding interaction of flavonoids such Naringin, quercetin, cyanidin and rutin were explored using ligand based approach to propose the inhibitory activity of flavonoids against IDO1 receptor.

2. Materials and Method

2.1 Ligand Preparation

The structure of flavonoid compounds were retreived from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and Chemspider database (http://www.chemspider.com/). The compounds were converted into comfortable format (.sdf) using Open Babel tool. The energy minimized structure were used for docking studies.

2.2 Protein Preparation

The three dimensional crystal structure of the target protein indoleamine 2,3dioxygenase 1 (IDO1) was retreived from Protein Data Bank (http://www.pdb.org/). The PDB ID for the selected target protein was 2D0T. The bonds, bond orders, explicit hydrogen, charges (calculated by MVD), flexible torsion and Tripos atom types for the protein were selected if any found omitted by using Protein Preparation, module of Molegro Virtual Docker for the protein Indoleamine 2,3 dioxygenase 1 (IDO1). The side chain flexibility analysis were performed which provides valuable insights to increase docking algorithms and grants an index of amino acid side chain flexibility, which potentiality aids in molecular docking. The atoms away from the binding site are neglected using ignored distant atom option. The active sites on the Indoleamine 2,3dioxygenase 1 were analysed using Molegro Virtual Docker.

2.3. Molecular docking

Molegro virtual docker works on the basis of Moldock SE search algorithm The docking algorithm was set at a maximum iteration of 1500 with a simple evolution size of 50 and minimum of 5 runs. The population size was set at 50 with energy threshold of 100 at each step. The least minute was set as 10 minutes, the torsions/translations/rotations of the ligand protein interaction were tested and the one giving lower energies is chosen for further studies. The bonds flexibility of the ligands were fixed and the side chain flexibility of the amino acids in the binding cavity was set with a tolerance of 1.10 and strength of 0.90 for docking simulations. RMSD threshold for multiple cluster poses was set at <2.00.

The reranking score function is estimated more expensive than the scoring function used during the docking simulation but it is commonly better than the docking score function at analyzing the best pose among several poses originating from the same ligand (Thomson et. al., 2006). Binding affinities were estimated using Molegro data modeler The scoring function used by MolDock is derived from the piecewise linear potential (PLP) scoring functions which further improves these score with a new hydrogen bonding term and new charge schemes (Thomsen et. al., 2006).

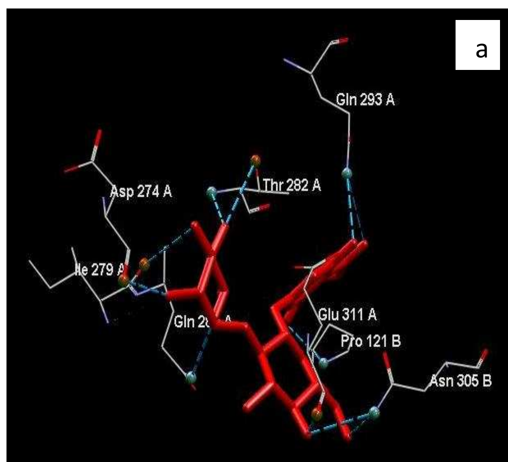
3. Results and Discussion

Natural products derived from medicinal plants and their synthetic derivatives have been used as a good lead molecule in case of cancer treatment (Anuf. AR et al., 2014). Docking analysis was carried out for the selected bioactive bioflavonoids against the target protein Indoleamine 2,3-dioxygenase (IDO 1) using Molgro docking software so as to evaluate the efficacy of these compounds in suppressing the activity of target protein. The Moldock score, Docking score and rerank score of the potent flavonoids were displayed in table.1.

Table-1: Moldock score for various flavonoids against target protein

S. No	Compound Name	Moldock Score	Rerank Score	Hydrogen Bond	Docking Score
1	Naringin	-171.737	-118.917	-17.536	-188.306
2	Hesperidin	-160.561	-123.559	-8.2606	-173.26
3	Chicoric Acid	-160.482	-127.888	-10.819	-172.505
4	Proanthocyanidins	-151.791	-39.651	-10.496	-158.988
5	Silymarin	-148.731	-120.675	-9.2821	-155.619
6	Rutin	-148.143	-20.1104	-13.576	-161.83
7	ß-Sitosterol	-140.559	-105.75	-2.5	-139.5761
8	Pterostilbene	-139.328	-115.607	-4.987	-141.352
9	Malvidin	-129.448	-113.741	-7.8314	-133.788
10	Capsaicin	-127.031	-88.3244	-5.4793	-131.923

The flavonoid compound Naringin showed more potent inhibitory activity against IDO 1 protein. Naringin is a naturally occurring plant bioflavonoid present in citrus fruits and possesses a wide range of biological application including anticancer. The interaction of the compound with the target protein was analysed using Molegro viewer module.



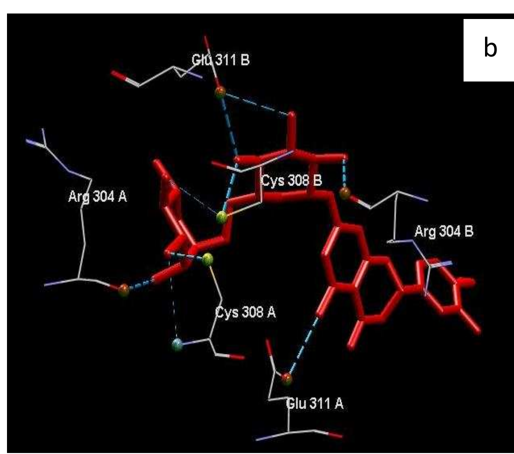


Figure. 1 a) Interaction of Naringin with IDO-1 protein, b) Interaction of Hesperidin with IDO-1 protein visualized using Molegro viewer.

The receptor bound ligand Naringin bound deeply in binding pocket region. The active compound Naringin bound with receptor with a moldock score of -171.737. The compound displayed interaction with seven different aminoacids with a total of 13 hydrogen bond interactions. Amino acid residue of IDO-1 involved in binding with bioactive flavonoids were displayed in Table 2

Table-2: Amino acid residue of IDO-1 involved in binding with bioactive flavonoids

S. No	Compound name	Hydrogen bond interaction with Amino acid	No. of Hydrogen bond
1	Naringin	Asp 274 A, Ile 279 A, Thr 282 A, Gln 283 A, Gln 293 A, Pro121B, Asn 305 B	13
2	Hesperidin	Arg 304 A, Cys 308 A, Glu 311 A, Arg 304 B, Cys 308 B, Glu 311 B	11
3	Chicoric Acid	Gln 293 A, Gly 284 B, Gly 286 B, Asp 294 B, Arg 296 B, Glu 311 B	9
4	Proanthocyanidins	Thr 282 A, Gln 293 A, Arg 304 A, Arg 304 B, Cys 308 B, Glu 311 B	8
5	Silymarin	Gln 293 A, Glu 311 A, Glu 119 B, Leu 120 B, Gln 293 B, Arg 296 B, Asn 305 B	8
6	Rutin	Lys 238 A, Gln 293 A, Arg 297 A, Thr 282 B, Gly 284 B, Ala 289 B, Gln 290 B, Gln 293 B, Asp 294 B	10
7	ß-Sitosterol	Gln 280 A, Glu 119 B	2
8	Pterostilbene	Gln 280 A, Arg 296 A, Arg 297 B	3
9	Malvidin	Thr 282 A, Gly 284 A, Gln 293 A, Glu 311A, Pro 121 B, Arg 297 B, Arg 304 B	8
10	Capsaicin	Gln 271 A, Asp 274 A, Thr 282 A, Glu 311 A	4

4. Conclusion

The present molecular docking studies provide insights into inhibition of IDO-1 by various bioactive flavonoids. Docking study propose that Naringin has a high binding affinity for IDO-1 protein. This study has led to the development of novel lead molecules which would help to develop enzymatic mechanisms allowing tumors to resist or escape immune rejection.

5. Reference

1. Anuf AR, Breethi Natarajan, Shahila Stephen and Kannan Ramaraj, Insilico Analysis of Plumbagin and its synthetic analogues as potential Bcl-2 Inhibitors, A. J. Med. Pharm. Sci., 2014, 2(2): 108-114.
2. Chahar MK, Sanjaya Kumar DS, Lokesh T, Manohara KP. Invivo antioxidant and immunomodulatory activity of mesuol isolated from Mesuaferrea L. seed oil. IntImmunopharmacol 2012; 13(4): 386–391.

3. De Jong RA, Kema IP, Boerma A, Boezen HM, van der Want JJ, Gooden MJ, Hollema H, Nijman HW, Prognostic role of indoleamine 2,3-dioxygenase in endometrial carcinoma. Gynecol Oncol. 2012 Sep; 126(3): 474-80
4. Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, Orabona C, Bianchi R, Belladonna ML, Volpi C, Santamaria P, Fioretti MC, Puccetti P. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naïve T cells. J Immunol 2006; 176:6753-61.
5. Gibellini L, Pinti M, Nasi M et al. Quercetin and cancer chemoprevention. Evid Based Complement Alternat Med 2011: 591356
6. Holden J, M.; Bhagwat, S.A., Patterson, K.Y. Development of a multi-nutrient data quality evaluation system.J. Food Comp. Anal. 2002; 15(4):339-348.
7. KannanRamaraj, Alexander Ronaldo Anuf, Inhibition of FGFR2 signal transduction in Acne Vulgaris using Bioactive Flavonoids: An Insilico approach,A. J. Med. Pharm. Sci., 2014, 2(2): 136-142
8. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor Al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998; 281(5380):1191-1193.
9. Soliman H, Mediavilla-Varela M, Antonia S. Indoleamine 2,3-dioxygenase: is it an immnune suppressor? Cancer J 2010; 16: 354-9
10. Thomsen R, Christensen MH. MolDock: A New Technique for High-Accuracy Molecular Docking. *J Med Chem.,* **2006**, 49(11): 3315
11. Vasilis P. Androutsopoulos, Athanasios Papakyriakou, Dionisios Vourloumis, Aristidis M. Tsatsakis, Demetrios A. Spandidos, Dietary flavonoids in cancer therapy and prevention: Substrates and inhibitors of cytochrome P450 CYP1 enzymes., PharmacolTher. 2010 ;126(1):9-20
12. Zou W, Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer, 2005; 5(4):263-274.

PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIOXIDANT ACTIVITIES OF ETHANOLIC LEAF EXTRACT OF *MIMOSA PUDICA*

Deepa. R[1], Selvakumar. K[2], Saravanan. R[3] & Krishnamoorthy. G[4]

[1]Research and development Centre, Bharathiar University, Coimbatore.
Department of Biochemistry, Madha Dental College and hospital, Kundrathur, Chennai.
[2]Dr. A. Ramachandran's Diabetes Hospitals, Anna Salai, Guindy, Chennai.
[3]Department of Biochemistry, Karpaga Vinayaga Institute of Dental Sciences, Madhuranthagam, Chennai.
[4]Department of Biochemistry, Asan Memorial Dental College and hospital, Asan Nagar, Chengalpet.
Corresponding Author: Krishnamoorthy. G (gunakrish_bio@yahoo.com.)

ABSTRACT

BACKGROUND: *Free radicals are major factor in the pathogenesis of many diseases like diabetes, cancer, cardiovascular diseases auto immune disease etc. The medicinal plants can be used as a source for drugs. The use of plant extract and phytochemicals can be of great importance in therapeutic treatment. The aim of the study was to evaluate the preliminary phytochemical constituents and in-vitro antioxidants activities of ethanolic extract of mimosa pudica leaves.* ***METHODOLOGY:*** *Mimosa pudica leaves was collected and dried under shade. Air dried and powdered leaf sample was extracted by using ethanol solvent. The antioxidant and free radical scavenging activity of ethanolic extract of mimosa pudica leaf was assessed by 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method ,the phytochemical study were assessed using suitable procedure.* ***RESULT:*** *The phytochemical analysis of mimosa pudica leaf extract shows presence of flavonoid, alkaloid, tannin, carbohydrates, steroids, glycosides.* ***CONCLUSION:*** *These data indicates antioxidant activity of mimosa pudica leaf extract and the phytochemical may be responsible for the therapeutic uses of this plant.*

1. INTRODUCTION

Traditional medicine plays a vital role in the treatment of various diseases. In traditional medicine, plants have been used for past decades in daily life to treat and prevent diseases. Plants are a major contributor for preparation of herbal drug due to their active constituents. Herbal medicine is the oldest form of medicine known to mankind using plants[1]. According to World Health Organization medicinal plants are defined as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs. The World Health Organization has been reported that 80% population in Asia and Africa use herbal medicine for primary health care[2].

The plant *Mimosa pudica* is a creeping annual or perennial herb. It was first described from Brazil by Carl Linnaeus in species *plantarum* in 1753. It belongs to family *Fabaceae* and sub family of *Mimosaseae.* In Latin it is called *Mimosa pudica.* The other name of this plant is humble plant, sensitive plant, touch me not plant, sleeping grass. It is called as Ajalikalika in Sanskrit, Lajawanti in hindi, Lajjabate in bengali, Hadergitte in kannada, Thotachchenunggi or Tottalvadi in tamil and Attapatti in telugu[3]. It is diffusely spreading, half-woody herb, with branched stem up to 1 meter long, sparingly prickly with numerous deflexed, bristly hairs. Leaves are bipinnate, pinnae 1-2 pairs, leaflets 10-20 pairs, linear, glabrous, 9-12 mm long and 1.5 mm wide and they are very sensitive, both pinnae and leaflets folding when touched. Heads are long- peduncled, solitary or 2 to 3 in each axil, about 1 cm in diameter. Pods are flat, slightly recurved, 1 to 2 centimeter long, with 3 to 5 one sided joints that fall away on maturity. Florets are red in the upper part with pink to lavender filament[4].

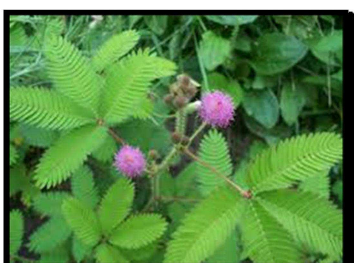


Fig.1 *Mimosa pudica*

Mimosa pudica is a well-known medicinal plant and it has been used in ayurvedha, unani and homeopathic medicine. Based on previous research, the *Mimosa pudica* leaf, root, shoot, seeds and whole plant showed medicinal property. *Mimosa pudica* has been reported that it possesses antibacterial[5], hepatoprotective[6], antioxidant[7], antidiabetic[8], anti-inflammatory[9], anticonvulsant[10], antimalarial[11], and antivenom12, antifertility and antiulcer activities[13]. The active constituents of medicinal plants are responsible for biological function and involved in the management of diseases which may include flavonoids, alkaloids, steroids, terpenoids etc. The presence of flavonoids and phenolic compound in plant extract, which possess very good antioxidant activity. These antioxidant activities help in the protection of human body against free radicals that may cause pathological disturbances. The phytochemical studies play a significant role in the identification of active constituents which is responsible for antioxidants activity.

The aim of the present study is to evaluate the phytochemical constitution and antioxidant activity of ethanolic extract of *Mimosa pudica* leaves.

2. MATERIALS AND METHODS

2.1 Collection of plant material

Fresh healthy, mature leaves of *Mimosa pudica* were collected from the wild population during in the month of December 2014 in Thiruvannamalai district. The leaves were washed thoroughly in sterile water and dried in shade, powdered and kept in an air tight container for further use.

2.2 Preparation of plant extracts

100 g of the powder of *Mimosa pudica* leaf was defatted with 500 ml of petroleum ether (60–80°C) overnight and it was then extracted in 80% ethanol by soxhalation, filtered and concentrated in a rotary evaporator. The dried extract (6% yield) was used for the present study.

2.3 Preliminary phytochemical analysis

The ethanolic extract of *Mimosa pudica* leaf was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures

2.3.1 Test for Alkaloids

Hager's test: To 2ml of leaf extract was taken in a test tube and few drops of Hager's reagent was added. The formation of yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test: The extract was mixed with 2 ml of Wagner's reagent. Formation of reddish brown coloured precipitate indicates the presence of alkaloids.

2.3.2 Test for Flavonoids

Alkaline Reagent test: The leaf extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute Hydrochloric acid indicates the presence of flavonoids.

2.3.3 Test for Steroids

Lieberman-Burchard's test: The leaf extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and 5 drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicated the presence of steroids.

2.3.4 Test for Carbohydrates

Molisch's test: The leaf extract was treated with 2 drops of Molisch's reagent in a test tube. 2 ml of concentrated sulphuric acid added along the sides of the test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

2.3.5 Test for Glycosides

Legal's test: The leaf extracts were treated with sodium nitroprusside in pyridine and sodiumhydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides

2.3.6 Test for Amino acid

Ninhydrin test: To the leaf extract, 10 drops of ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

2.3.7 Test for Tannins

Braymer's test: 2ml of extract was added to 2ml of water. To this 2-3 drops of 5% ferric chloride was added. Mix the reagent mixture gently and the formation of green precipitate indicates the presence of tannins.

2.3.8 Test for Terpenoids

Salkowski test: 5 ml of leaf extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown precipitate was not observed, absence of terpenoids.

2.3.9 Test for Saponins

Froth Test: The leaf extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. 1 cm layer of foam was not observed, absence of saponins.

2.4 In vitro antioxidant activity

The DPPH radical scavenging method was used to evaluate the antioxidant property. The concentrations of the plant extracts required to scavenge DPPH showed a dose dependent response.

2.4.1 Dose-dependent effect of total antioxidant activity

The total antioxidant activity was measured using thiocyanate method[14]. Dose (25, 50, 75 and 100mg/ml) dependent activity of extract was studied by preparing extract in ethanol. Then the leaf extract was added to 2.5ml of 40mM linoleic acid emulsion (pH 7.0) and 2ml of 40mM phosphate buffer (pH 7.0). The final volume was adjusted to 5 ml using 40mM phosphate buffer. Samples were then incubated at 37°C in a glass flask for 60h, to accelerate the oxidation process. 1ml of the incubated sample was removed after 12h, to which 0.1ml of 20mM ferric chloride and 0.1ml 30% ammonium thiocyanate were added.

The absorbance of this was measured at 500 nm, with BHA as the reference compound. Solvent was used as a test control to eliminate the solvent effect. All data reported are the average of triplicate analysis. % inhibition of lipid peroxide (LPO) generation was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Control OD - Test OD}}{\text{Control OD}} \times 100$$

2.4.2. Dose dependent effect of free radical scavenging activity

The radical scavenging activities of mimosa leaf extract were measured by DPPH method[15]. The following concentrations of extract were prepared 25, 50, 75 and 100mg/ml in ethanol. BHA used as an antioxidant standard. The extract was mixed with 1 ml of methanolic solution of DPPH (0.2 mM). The reaction mixture was vortexed and incubated for 30 min. The optical density of the solutions was measured at 520nm using BHA as the standard reference. The radical scavenging activity was calculated using the following formula.

Radical Scavenging Activity % =

$$\frac{\text{Control OD - Test OD}}{\text{Control OD}} \times 100$$

The unit of the total anti-oxidant activity is expressed as % inhibition of DPPH free radical.

2.4.3. Statistical Analysis:

Result were expressed as mean ± standard deviation (SD).Statistical comparison were made using the student t- test by one way analysis of variance (ANOVA). Significance difference was expressed in groups, in which a represents 25 mg/ml Vs other groups, b represents 50 mg/ml Vs other groups, c represents 75 mg/ml Vs other groups.

3. RESULT AND DISCUSSION

3.1 Preliminary phyotochemical analysis

The result of phytochemical analysis is shown in table 1. The ethanolic leaf extract of *Mimosa pudica* shown the presence of phytoconstituents include alkaloids, flavonoids, steroids, carbohydrate, protein, glycosides and tannin, saponnin and terpinoids was absent. In the previous study the methanolic extract of *Mimosa pudica* leaf and root used for phytochemical analysis. They reported that the leaf contains tannin, protein and steroids and the root contains tannin and protein[16].

Table 1. Phytochemical analysis of *Mimosa pudica* leaf extract

S. No.	Test	Phytochemicals	Result
1.	Hager's Test	Alkaloids	+
2.	Alkaline Reagent Test	Flavinoids	+
3.	Lieberman-Burchard's Test	Steroids	+
4.	Molisch's Test	Carbohydrates	+
5.	Legal's Test	Glycosides	+
6.	Ninhydrin Test	Aminoacids	+
7.	Braymer's Test	Tannins	+
8.	Salkowski Test	Terpinoids	-
9.	Froth Test	Saponin	-

+= Present and - = Absent

The flavonoids in *Mimosa pudica* have been reported their antibacterial activity[16].In another phytochemical study shown the presence of alkaloid non protein amino acid called mimosine which possess antidermatophytic and antibacterial activity[17].The tannin isolate from *Mimosa pudica* root was reported their anti-snake venom activity[18].

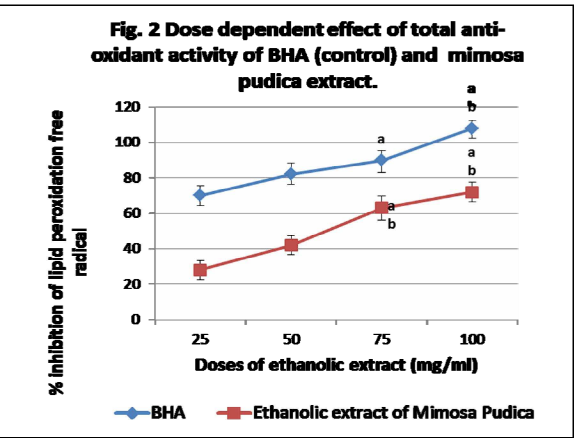
Alkaloids from natural herb exhibit anti-cancer activity it may include berberine, evodiamine, piperine and matrine.[19]The plant *Mimosa pudica* have been reported their anticancer, antiulcer and antidiabetic activity due to presence of active flavonoids and phenolic compound. Two molecules of C-glycosyl flavones have been identified in previous study of *Mimosa pudica*[20]. The phytochemical studies needed to identify the active compound which is present in medicinal plants. It is helpful for creating new therapeutic strategy for prevention and treatment of various diseases.

3.2 In vitro antioxidant activity

3.2.1. Dose dependent effect of total antioxidant activity

In the present study, the antioxidant activity of mimosa pudica leaf extract was determined by thiocyanate method. The result of total antioxidant activity of ethanolic extract of *Mimosa pudica* is shown in figure 2. The phytoconstituent like flavonoids and tannins have antioxidant activity which scavenge the free radicals. In the previous study it was suggested that phenolic component and flavonoids are major component in the plant which possess antioxidant activity[21]. In the present phytochemical studies shows that the *Mimosa pudica* leaf extract contains flavonoids, steroids and tannin and glycosides. The presence of these active constituents scavenge free radicals due to presence of more hydroxyl groups[22].

The different concentrations of ethanolic extract of Mimosa pudica 25, 50, 75,100 mg/ml showed peroxidation inhibition in a dose dependent manner. In the present study, Mimosa pudica leaf extract at concentration 100 mg/ml showed maximum inhibition of peroxide radical near control BHA.



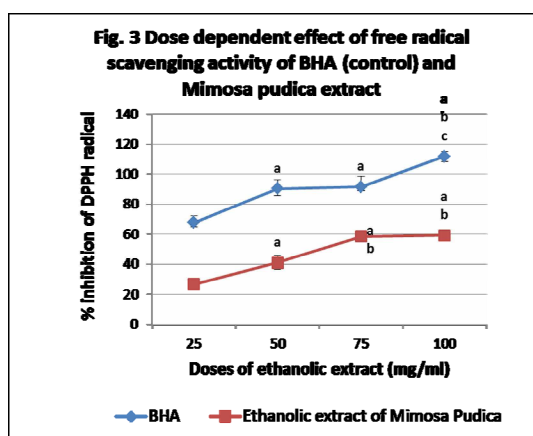
Result were expressed mean ± SD. Statistical comparison were made using student t-test by one way analysis of variance. Significant difference was expressed in groups. Whereas a = 25 mg/ml vs other goups, b = 50 mg/ml vs other groups, c = 75 mg/ml vs other groups.

The result indicates that the ethanolic extract of *mimosa pudica* leaf significantly inhibited linoleic acid peroxidation.

3.2.2. Dose dependent effect of free radical scavenging activity

The free radical scavenging activity of extract was detected by DPPH method. DPPH is a free radical and accept the hydrogen radical to become a stable molecule. It is used as a substrate for detection of antioxidant activity of extract. The result of free radical scavenging activity of *Mimosa pudica* extract is shown in figure 3. The ethanolic extract of *Mimosa pudica*

leaves showed DPPH scavenging activity and BHA used as control.



Result were expressed mean ± SD. Statistical comparison were made using student t-test by one way analysis of variance. Significant difference was expressed in groups. Whereas a = 25 mg/ml vs other goups, b = 50 mg/ml vs other groups, c = 75 mg/ml vs other groups.

The DPPH scavenging activity increased by increasing concentration of leaf extract due to the presence of flavonoids and phenolic compound which having hydroxyl groups. The antioxidant has ability to reduce stable radical to 1-1, diphenyl-2-picryl hydrazine and the degree of discolouration indicates the scavenging activity of the extract. The maximum inhibition observed at the concentration of 100 mg of mimosa leaf extract.

The peroxidation of lipids initiated by free radicals may lead to cell injury. Free radicals are molecule with one or more single pair of electron that can quickly react with the constituents like proteins, nucleic acid, lipids etc. Antioxidant may offer resistance against oxidative stress by scavenging free radicals. Several research on antioxidant activity of plant reported flavonoids are powerful antioxidant against free radicals. It inhibits the lipid peroxidation in vitro in initial stage and terminates the chain radical reaction by donating hydrogen atom. The flavonoids which present in mimosa pudica are isoquercetin, avicularin, apigenin-7-O--D-glucoside, cassiaocidentalin B, orientin, and isoorientin[23]. Gossypium herbaceam have been investigated their antioxidant and acetylcholine esterase inhibition, the result suggest due to presence of Quercetin, isoquercetin and quercemeritin flavonoid which possess antioxidant activity[24].

In another research the plant canarium album have been investigated their antioxidant activity, the result shown that it has good antioxidant activity due presence of tannin. Tannin extracted from canarium which shows very good DPPH radical scavenging activity[25].So the phytoconstituents are major contributor for scavenging free radicals.

CONCLUSION

Phytochemical screening of ethanolic extract of mimosa pudica shows the presence of alkaloids, flavonoids, tannin, glycosides, steroids, protein and carbohydrate. The result indicates that mimosa pudica leaf extract have antioxidant activity or radical scavenging property due to presence of these active phytocostituents. The plant have potential source for therapeutic purpose however further study on isolation and characterization of antioxidant constituents is required.

REFERENCES

1. Baby Joseph, Jency George, Jeevitha Mohan. Pharmacology and traditional uses of *Mimosa pudica*. International journal of pharmaceutical sciences and drug research. 2013; 5(2):41-44.
2. Uddipon Aziz, Rumana Akther, Mohammad Shahriar and Mohiuddin Ahmed Bhuiyan.In vivo pharmacological investigation of *Mimosa pudica* L.Int J Pharm Sci 2013; 6(2):66-69.
3. Lubna Azmi, Manish Kumar Singh and Ali Kamal Akhtar. Pharmacological and biological overview on *Mimosa pudica* Linn. International journal of pharmacy & life sciences. 2011; 2(11)1226-123.
4. Srikanda Chowdhary, DiyajyotiSaha and Swati Paul. In vitro cytotoxic activities of methanolic extract of *Mimosa pudica*. Bulletin of Pharmaceutical research 2012; 2(1): 42-5.
5. Doss A, Vijayasanthi M, Parivuguna V and Anand P. Evaluation of antibacterial properties of ethanol and flavonoids from *Mimosa pudica*linn and panicum maximum jacq. Plant Science Feed 2011; 1(2):39-44.

6. Rekha Rajendran, Hemalatha S, Akasakalai K, MadhuKrishna C H, Bavan Sohil, Vittaland Meenakshi Sundaram R. Hepatoprotective activity of *Mimosa pudica* leaves against Carbontetrachloride induced toxicity. Journal of Natural Products. 2009:116-122.
7. Zhang J, Yuan K, Zhou W, Zhou J and Yang P. Studies on the active components and antioxidant activities of the extracts of *Mimosa pudica* Linn from southern China. Pharmacognosy Magazine 2011; 7(25): 35-39.
8. Sutar N G, Sutar U N, Behra B C. Antidiabetic activity of *Mimosa pudica* linn in albino rats. Journal of herbal medicine and toxiclogy.2009;3(1):123-126.
9. Pradeep Kumar Vikram, Reetesh Malvi, Deepak Kumar Jain. Evaluation of analgesic and anti-inflammatory potential of *Mimosa pudica* linn. Int J Pharm Res. 2012; 4(4): 47-50.
10. Ngo Bum E, Dawack D L, Schmutz M, Rakotonirina A, Portet C, Jeker A, Olpe H R and Herrling P. Anticonvulsant activity of *Mimosa pudica* decoction.2004;75(3):309-314.
11. Aarthi N, Murugan K. Antimalarial activity and phytochemical screening of ethanolic leaf extract of phyllanthusniruri and *Mimosa pudica*. IJPRD. 2011; 3(3)24: 198-205.
12. Monimala Mahanta, Ashis Kumar Mukherjee. Neutralisation of lethality, mytotoxicity and toxic enzymes of najakaouthia venom by *Mimosa pudica* root extracts. 2001;75(1):55-60
13. Elango V, Carolin Oliver and Raghu P S.Antiulcer activity of the ethanolic extract of *Mimosa pudica* in rats. Hygeia J.D. Med. 2012; 4(1):34-40.
14. Mitsuda, H., Yuasumoto, K., and Iwami, K. (1996) Anti oxidation action of indole compounds during the autoxidation of linoleic acid. Eiyo to Shokuryo 19. 210-214.
15. Braca A, Sortino C, Politi M, Morelli I, Mendez J.Antioxidant activity of flavonoids from Licania licaniaeflora. J Ethnopharmacol. 2002; 79:379-381.
16. Ranjeet Kumar Ranjan, Sathish Kumar M, Seethalakshmi and Rao. Phytochemical analysis of leaves and roots of *Mimosa pudica* collected from kalingavaram, Tamil Nadu. Journal of pharmaceutical research. 2013, 5(5): 53-55.
17. Doss A, Vijayasanthi M, Parivuguna V and S.P. Anan. Evaluation of Antibacterial properties of Ethanol and Flavonoids from *Mimosa pudica* Linn. and Panicum maximum Jacq. Plant sciences Feed 2011;1(2):39-44.
18. Anitha R, Jayavelu S, Murugesan K. Antidermatophytic and bacterial activity of mimosine. Phytother Res. 2005; 19(11): 992-3.
19. Ambikabothy J, Ibrahim H, Ambu S, Chakravarthi S, Awang K, Vejayan J. Efficacy evaluations of *Mimosa pudica* tannin isolate (MPT) for its anti-ophidian properties. Journal of Ethanopharmacology. 2011, 137: 257-262.
20. Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan P T, Deccaraman M, Vijayalakshim M, Dhamotharan, Dananjeyan B and Sathya Bama. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. Journal of Medicinal Plants Research. 2010; 4(12): 1089-1101
21. Anjali Soni and Sheetal Sosa.Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extract.Journal of pharmacognosy and phytochemistry. 2013; 2(4): 22-29.
22. Jin-Jian Lu, Jiao-Lin Bao, Xiu-Ping Chen, Min Huang, and Yi-Tao Wang.Alkaloids Isolated from Natural Herbs as the Anticancer Agents. Volume 2012 (2012), Article ID 485042, 12 pages.
23. Harleen Kaur Sandhar, Bimlesh Kumar, Sunil Prasher, Prashant Tiwari, Manoj Salhan, Pardeep Sharma. A Review of Phytochemistry and Pharmacology of Flavonoids. Internationale Pharmaceutica Sciencia. 2011; 1(1): 25-41.
24. Yongxin Zhao, Jun Dou, Tao Wu and Haji Akber Aisa. Investigating the Antioxidant and Acetylcholinesterase Inhibition Activities of Gossypium herbaceam. Molecules 2013, 18, 951-962.
25. Liang-liang Zhang and Yi-ming Lin. Tannins from Canarium album with potent antioxidant activity. J Zhejiang Univ. Sci. 2008; 9(5): 407-415.

GROWTH AND CHARACTERIZATION OF TRYPTOPHAN DOPED WITH PARACETAMOL

G. Madhurambal[1] & B. Kavitha[2]
1 Dean of Sciences and HOD of Chemistry,
A.D.M. College for Women, Nagapattinam – 611001.
Email: madhumaniam@yahoo.com
2 Assistant Professor in Chemistry, TBML College, Porayar- 609307
Email: kavithanadarajan@rediffmail.com

ABSTRACT

In Pharmaceutical field, the search for compounds that have suitable properties to be used in nowadays a big challenge, paracetamol, one of the most widely used drug is taken and the effect of dopant Tryptophan on the growth process, crystalline properties of paracetamol are investigated. The grown crystal was transparent, obtained within a period of 10 days. The grown crystal was subjected to various studies, such as X – Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and Ultraviolet – Visible (UV- Vis) absorbance, Thermogravimetric analysis and Differential thermal analysis (TGA / DTA). The functional groups of the crystals have been confirmed by FTIR analysis. The lattice Parameters of the grown crystal were determined by Single Crystal XRD. A UV- Vis spectrum was recorded to find the suitability of the crystal for optical applications and band gap energy is determined. The thermal stability of doped crystal was checked using the TGA / DTA analysis.

Keywords: *Crystal growth, FTIR, UV – VIS, Thermogravimetric analysis, XRD.*

1. Introduction

In the last years, many leading pharmaceutical companies have begun to strictly control the crystal chemistry of active pharmaceutical ingredients during their preparation and development stages. In several cases, the presence, or the discovery, of new crystal phases, initially interpreted as annoying side effect, was positively used to tailor new synthetic processes and more efficient formulation of drugs (1). Crystals are the pillars of modern technology. Crystals of different materials have several applications. Hence industry has taken interest in the field of crystal growth. In early days, mineral crystals were available to meet requirements, but now the natural source is found to be quite inadequate. To solve this acute problem, the synthesis of the material in the form of crystal is necessary (2). Paracetamol (PCT) is an arylated aromatic amide, which was firstly introduced into medicine as an antipyretic / analgesic by von Mering in 1893 and has been in use as an analgesic for home medication for over thirty years and is accepted as a very effective treatment for the relief of pain and fever in adults and children. It is the most used medicine after acetylsalicylic acid in many countries as an alternative to aspirin and phenacetin (3,4). Amino acids and their complexes belong to a family of organic materials that have been considered for photonic application (5).In recent years, the amino acid group materials were mixed with organic(6) or Inorganic salts (7) in order to improve their chemical stability, thermal stability, linear and non- linear optical properties. The dopant Tryptophan is an amino acid. As no reports are available on the growth and characterization of Tryptophan doped paracetamol crystals, in this work, we report on the growth and characterization of Tryptophan doped Paracetamol crystals by slow evaporation technique.

2. Experimental Methods

Pure paracetamol and Tryptophan was purified by successive recrystallization. Using doubly distilled water, the supersaturated paracetamol was prepared. To this, the dopant Tryptophan of 0.1M concentration was added. The solution was filtered with a micro filter and left for slow evaporation at room temperature. The crystallisation took place within 10 days and the optically transparent crystals of Tryptophan doped paracetamol were obtained.

3. Result and Discussion

3.1 Single Crystal X-ray Diffraction

The single crystal X-ray diffraction analysis on Tryptophan doped paracetamol single crystals was recorded using X-ray diffractometer. This analysis has revealed that the single crystals of doped paracetamol crystallize in monoclinic system. The calculated lattice parameters for doped paracetamol are compared with that of pure paracetamol(5) and are given in Table 1. The XRD analysis revealed that the addition of dopant in the paracetamol does not change the crystal structure though there is a enormous change in the lattice parameters.

Table 1: comparison of unit cell parameters

	Pure Paracetamol	Doped Paracetamol
a (A^0)	11.72 A^0	7.101 ± 4.619
b (A^0)	9.7379 A^0	9.410 ± 0.329
c (A^0)	7.109. A^0	11.79 ± 4.681
system	monoclinic	monoclinic
volume	774.89 A^{03}	774 A^{03}

3.2 FTIR Analysis

The FTIR spectra for pure as well as doped paracetamol crystals were recorded using FT-IR instrument using the KBR pellet technique in the range 400 – 4000 cm $^{-1}$ is shown in the figure 1. The calculated frequencies with their relative intensities obtained in FTIR of pure and doped paracetamol and their most probable assignments are presented in Table 2.

Table 2: Vibrational frequencies obtained for pure and doped paracetamol

Observed IR Frequencies		Assignments
Pure Paracetamol	Doped Paracetamol	
3928.22	3897.04	OH stretching
-	3403.26	Inter molecular hydrogen bonded OH stretching
3323.82	3325.86	NH stretching
3161.66	3039.41	NH stretching associated, Intra molecular hydrogen bonded OH stretching
2881.27	2818.77	Symmetric CH stretching, Intra molecular hydrogen bonded OH stretching
2713.47	2718.84	Intra molecular hydrogen bonded OH stretching
2586.77	2578.84	
2038.09	2075.20	Overtone C-H out of plane bending and combination band
1978.55	-	
1877.49	1883.08	
1654.66	1658.50	C=O stretching
1565.00	-	NH deformation, aromatic C=C stretching
1507.78	1510.60	NH deformation, aromatic C=C stretching
1438.62	1415.79	Asymmetric C-H bending, OH in-plane bending
1367.09	1356.67	Symmetric C- H bending, OH in-plane bending
1325.50	-	C- N stretching
1229.60	1231.38	C- O bond
-	1155.83	NH in - plane bending vibration in heterocyclic compound
966.80	920.99	Asymmetric ring stretching
835.52 802.20	840.66 803.55	Aromatic C – H out- of-Plane bending
716.47	742.31	Aromatic C – C out- of-Plane bending, OH out-of-plane bending
683.06	683.02	
601.38	625.66	OCN bending vibration
- -	586.78 557.03	NH out- of- Plane bending vibration

1. The frequency assignments of the Tryptophan doped paracetamol depicted that the Tryptophan entity approaches the paracetamol molecule just by forming hydrogen bond between amino group of Tryptophan and OH group of paracetamol and carboxylic end forming hydrogen bond with acetylamido end inverting the heterocyclic ring away from Benzene entity. This is supported by the appearance of the

frequencies at 3403.26cm^{-1} ,1155.83 cm^{-1}, 586.78 cm^{-1} and 557.03 cm^{-1} are due to intermolecular hydrogen bonded OH stretching, NH in- plane bending vibrations and NH out-of-plane bending vibrations respectively in the Tryptophan doped paracetamol FTIR. This is further supported by large decrease in the frequencies region 3928.22 cm^{-1},3161.66 cm^{-1} and 2881.27 cm^{-1} which are due to the intermolecular hydrogen bonded OH stretching frequencies. The frequencies in the region 1978.55 cm^{-1} , 1565.00 cm^{-1} and 1325.50 cm^{-1} which are due to CH out-of-plane bending, aromatic C=C stretching and C-N stretching vibration of paracetamol are absent in the Tryptophan doped paracetamol spectra which may be due to the hydrogen bonded attachment of NH_2 and COOH entities to paracetamol so that the stretching behavior of the aromatic ring is curtailed to some extent. This is also supported by the minor variations in the C=O stretching aromatic C=C stretching, aromatic CH out-of-plane bending in the region 1613.05 cm^{-1},1507.78 cm^{-1},835.52 cm^{-1} and 802.20 cm^{-1} respectively. As the bulky group approach through two ends to the paracetamol molecules the acetyl group may form intramolecular hydrogen bond with C-O oxygen to the ortho hydrogen of the aromatic ring of the paracetamol. There is also variation in the asymmetric CH bending and symmetric CH bending of the methyl group in the region 1438.62 cm^{-1} and 1367.09 cm^{-1} respectively.

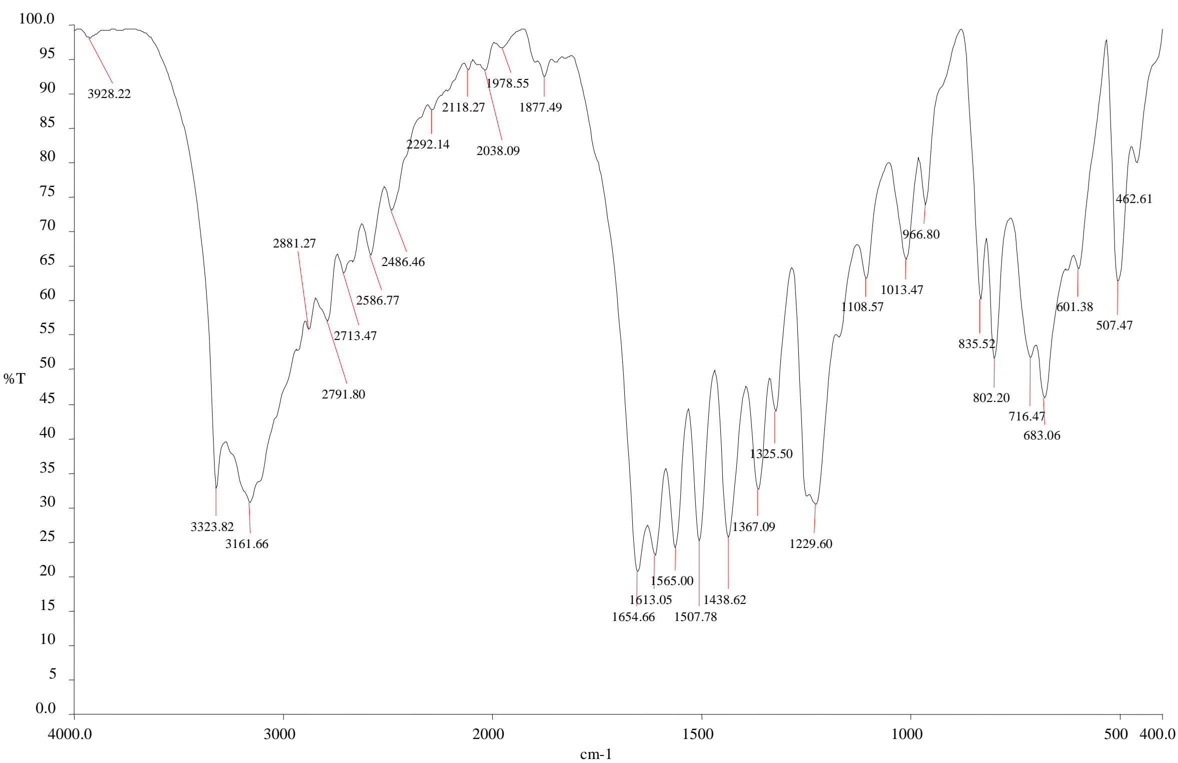


Figure -1 FTIR – spectrum of pure paracetamol.

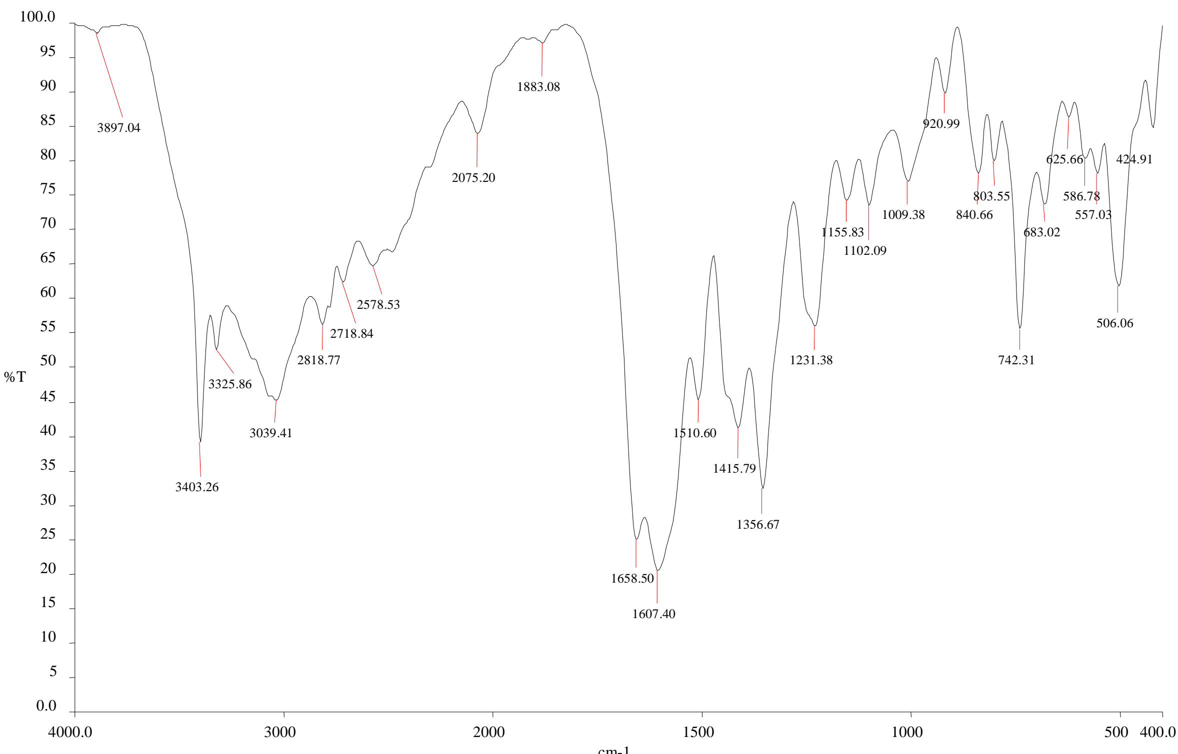


Figure -2 FTIR – spectrum of doped paracetamol

3.3 UV – VIS spectral studies

UV-Visible spectral study is a useful tool to determine the transparency, which is an important requirement for material to be optically active (8). The pure paracetamol and Tryptophan doped paracetamol was recorded in the range of 190 – 1100 nm using lambda 35 spectrometer and shown in the figure (2).

The electronic absorption of the paracetamol and Tryptophan doped paracetamol has cut off wavelength in the region 320nm. As there is no considerable change in the cut off wavelength due to the addition of Tryptophan and hence for both UV and visible region the paracetamol and Tryptophan doped paracetamol can be used as an optical window. Regarding the electronic absorption the corresponding n-π* transition of the amide linkage is very much shifted and undergoes bathochromic shift due to addition of the dopant ,and at the same time the absorption frequency corresponding to hydroxy linkage is not shifted to higher frequency region which supports occupancy Tryptophan closer to amide linkage in paracetamol.

.

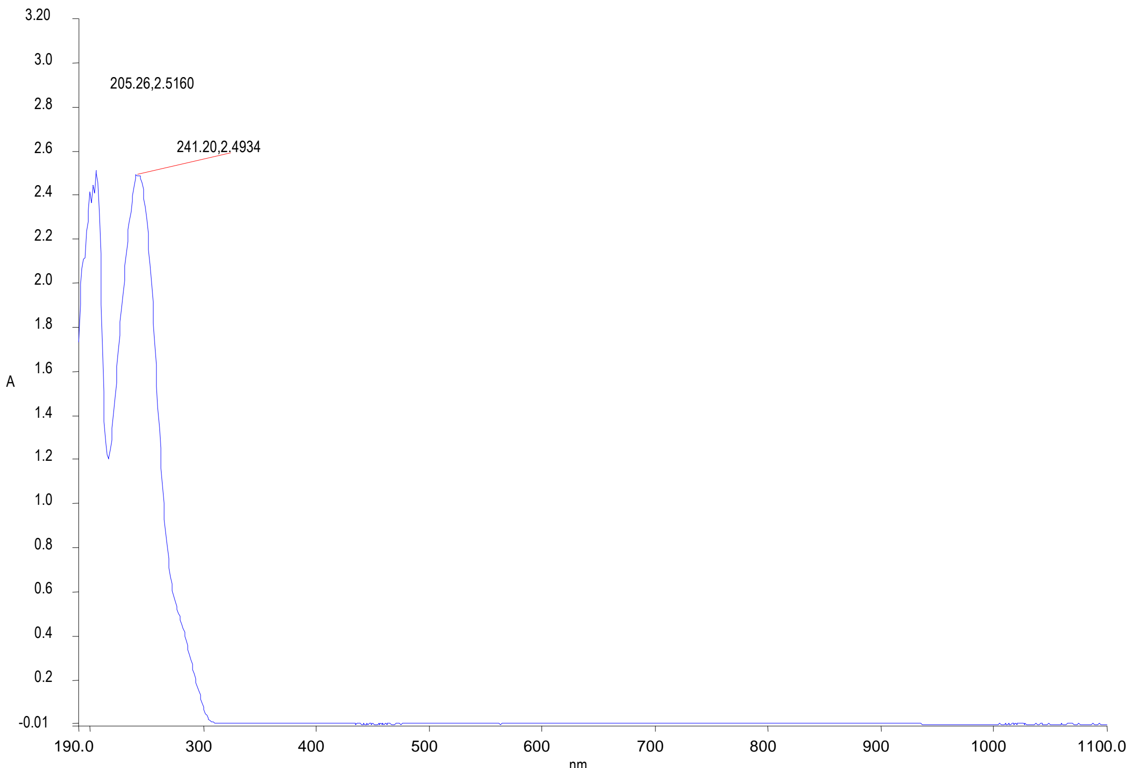


Figure - 3 UV – Visible spectrum of pure paracetamol

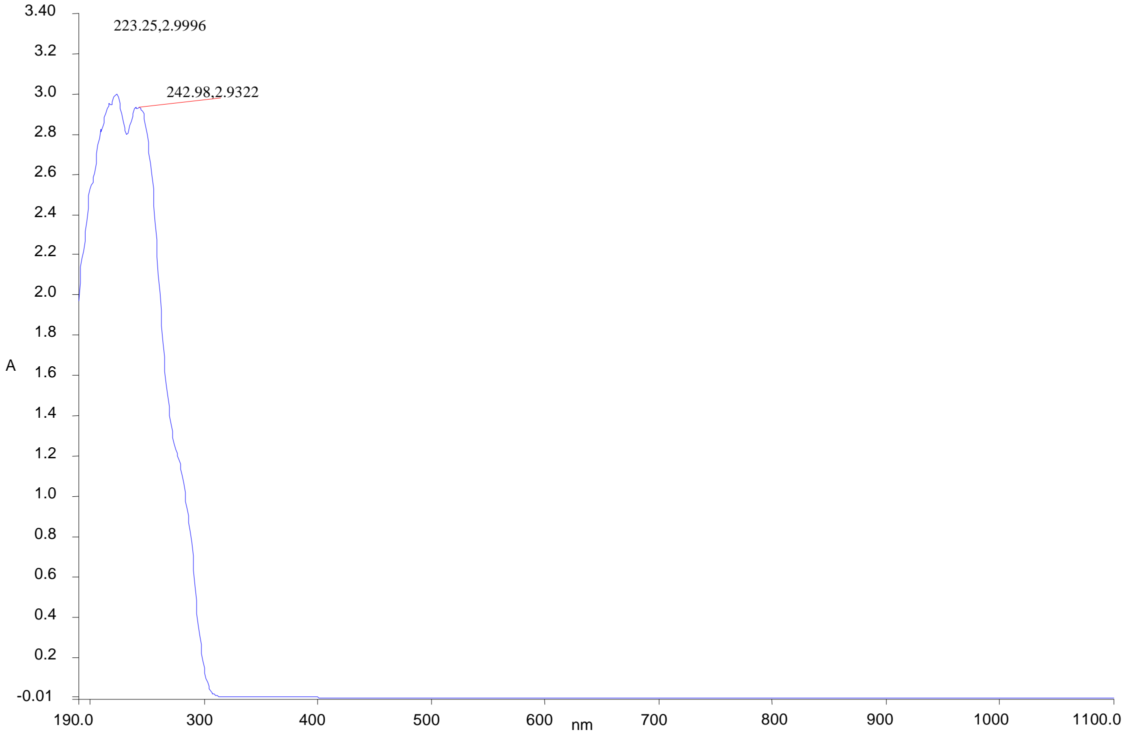


Figure :4UV – Visible spectrum of doped paracetamol.

3.4 Band gap determination

The optical band gap of the paracetamol and tryptophan doped paracetamol was determined from the absorption spectrum using the near-band absorption relation.

$$(\alpha h \gamma)^n = A (h\gamma - Eg)$$

Where A - the optical transition dependent constant, Eg – optical energy band gap , γ – the frequency of incident beam, h – planck's constant, n – characterizes the transition. Fig (5,6) shows the plot of $(\alpha h \gamma)^2$ against hγ. The intercept of the straight line on the photon energy axis give the direct band gap value of 4.680ev for paracetamol and 4.56ev for doped paracetamol. Due to the addition of dopant, the band gap narrowing occurred.(9)

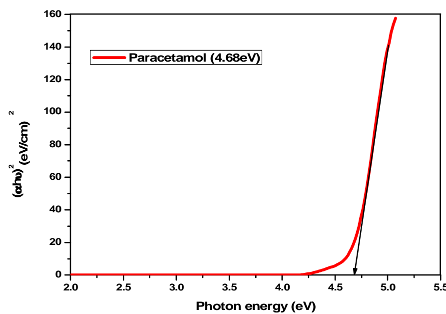


Figure :5 Band Gap Energy of Paracetamol

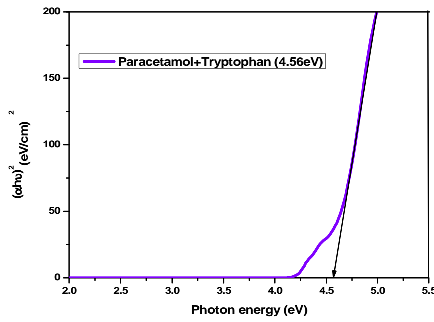


Figure :6 Band Gap Energy of doped Paracetamol

3.5 Thermal Analysis

Theromogravimetric analysis (TGA) is a technique in which the weight of a substance is recorded as a function of temperature.(10,11) In the present case, the TGA and DTA are carried out between 30°c and 930°c in the nitrogen atmosphere which provide an inert environment is shown in the figure (7).In the first step, the weight loss started at 125°c and completed at 320°c. At the first stage about 90.73% of weight loss occurred which is assigned to the loss of acetanilide, Indole, Acetate anion and carbon residue.At the second stage of 450°c, there is one more weight loss of 5.074% due to the loss of H_2 O- In the final step there is 3.875% due to the loss of ammonia group.

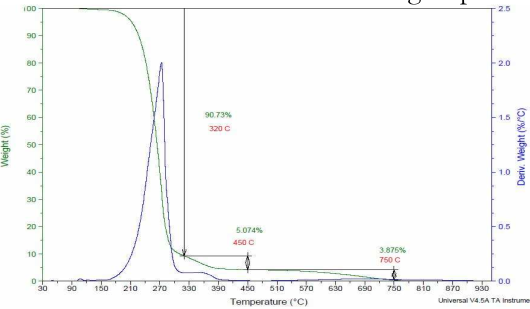


Figure: 7 TGA of Doped Paracetamol.

3.6. Optical Image Microscope:

The crystals are photographed using optical Microscopy LX400. The pure paracetamol and doped paracetamol are colorless and transparent crystals. The photographs of the crystals are shown in the figure (8,9).

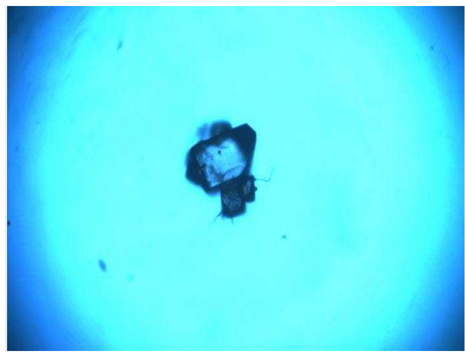


Figure :8 optical image of pure paracetamol.
Figure :9 optical image of doped paracetamol.

4. Conclusions

Good optical quality single crystals of pure and doped paracetamol have been grown by slow evaporation technique under room temperature. The doped crystals were characterized by single crystal X-ray difrraction and confirmed that the crystal belong to monoclinic system. The presence of the functional groups and the presence of dopants were determined qualitatively using FTIR analysis. The UV absorption studies revealed that the doped paracetamol has been used as a optical window. TA – DTA studies revealed the thermal stability of the crystal. Band gap determination revealed the addition of dopant narrowing the band gap. With promising structural, optical and thermal properties, Tryptophan doped paracetamol single crystals are a potential material for frequency conversion device applications.

References

1. R.Hilfiker, Polymorphism in the pharmaceutical Industry Wiley. VCH, 2006.
2. P.Santhanaragavan, P.Ramasamy, Crystal Growth processes and methods, KRU Publications, Kumbakonam, 1998.
3. M.Espinosa Bosch, A.J.Ruizsanchez, F.Sanchez Roias, C-Bosch Ojeda. Determination of paracetamol: Historical Evolution, Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 291 – 321
4. M.I Walash, S.P. Agarwal and M.I. Martin, can-J.Pharm – Sci., 1972, 7, 123.
5. JCPds ID NO. 39 – 1503
6. Y.R.Sharma, Elementary organic spectroscopy, principles and chemical applications, First Edition, 72 – 133 (1980)
7. Jag Mohan,Organic spectroscopy, principles and applications, second Edition, 18 – 104, 2000;
8. V.Venkatramanan, S.Maheswaran, J.N.Sherwood, and H.L.Bhat, "Crystal growth and physical characterization of the semi organic bis(thiourea) cadmium chloride "Journal of Crystal Growth, Vol.179, no. 3-4, PP.605-610, 1997.
9. Sunil chaki, M.P.Wesh Pande, Jiten P. Tailor, Mahes D.Chaudhary, Kanchan Mahato, Growth and Characterisationof ADP single crystal," American Journal of condensed Matter Physics 2012, 2(1): 22 – 26.
10. Gopalan, R.etal, Elements of Analytical Chemistry, Second Edition, 171-183, 199-216. (1994).
11. H.M.Patil, D.K.Sawant, D.S .Bhavsar, J.H.Patil and K.D.Girase. J. Therm. Anal. Chem., 2001.

MOLECULAR DOCKING STUDIES OF PHYTOCONSTITUENTS FROM *ALLIUM SATIVUM* AGAINST GLYCOGEN SYNTHASE KINASE 3B PROTEIN

Radhika.S, Ramaraj.K and *Ronaldo Anuf.A**

Department of Biotechnology, Kamaraj College of Engineering and Technology
Virudhunagar, Tamilnadu
Corresponding Email id: ronaldoanuf@gmail.com

ABSTRACT

Allium sativum is an important ayurvedic herbal plant used for the treatment of wide spectrum of diseases including cancer and diabetes.The bulbs of the plant have been used in many as a stimulant, antiseptic, anthelminthic, diuretic and for the relief of rheumatic pains. The plant possess significant antioxidant, antibacterial and anti-inflammatory activity. The present study focuses on exploring novel drug lead molecules from allium sativum as potential inhibitors of Glycogen synthase kinase 3β protein, an effective wound healing biomarker. GSK3-β is involved in cell development, and body pattern formation, whose inhibition promotes wound healing through β-catanin-dependent Wnt signalling pathway. The molecular docking analysis is performed using Molgro docking software. Of the different phytoconstituents selected for study the compound alliin revealed high binding activity with a Mol Dock score of -70.06.The docked pose of the compound allicin fits exactly at the active site with maximum number of hydrogen bond interactions.

Keywords: *Allium sativum , Molegro, Glycogen synthase kinase 3β*

1. Introduction

The initial characterization glycogen metabolism regulator is Glucogen Synthase Kinase – 3 (GSK – 3), which was the family of protein serine kinases (Plyte, S. et. al., 1992). The glycogen synthase residues are phosphorylated by the protein kinase and the dephosphorylation of the rate-limiting enzyme of glycogen synthesis in response to insulin (Parker P et. al., 1983). In mammals, two closely related proteins such as GSK-3α and GSK-3β, which are coded by two different genes.GSK-3β consist of 420 amino acids and the phosphorylation of tyrosine-256 is required for the activation (Wang et. al., 1994). It is involved in body pattern formation, neuronal cell development and energy metabolism.

Allium sativum (Garlic) is natural plant being used as a food as well as folk medicine for centuries all over the world. Garlic is reported to possess potent biological properties like antimicrobial, anti-cancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic, and anticardiovascular effects (Reuter HD, 1996). Different garlic extracts have demonstrated potent activity against Gramnegative and Gram-positive bacteria including species of *Escherichia*, *Salmonella, Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *Clostridium*, *Helicobacter pylori* and even acid-fast *bacilli* (AFB) such as MTB (Uchida Y, 1975).

In this study, we aim at exploring novel phyto-constituents from *A.sativum*as potential lead molecules against the target protein Glucogen Synthase Kinase – 3β (GSK-3β) using Molegro Virtual Docker.

2. Materials and Method

2.1 Ligand Preparation

The structure of flavonoid compounds were retrieved from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and Chemspider database (http://www.chemspider.com/). The compounds were converted into comfortable format (.sdf) using Open Babel tool. The energy minimized structure were used for docking studies.

2.2 Protein Preparation

The three dimensional crystal structure of the target protein Glucogen Synthase Kinase – 3β (GSK-3β) was retreived from Protein Data Bank (http://www.pdb.org/). The PDB ID for the selected target protein was 3GB2. The bonds, bond orders, explicit hydrogen, charges (calculated by MVD), flexible torsion and Tripos atom types for the protein were selected if any

found omitted by using Protein Preparation, module of Molegro Virtual Docker for the protein Glucogen Synthase Kinase – 3 (GSK-3β). The side chain flexibility analysis were performed which provides valuable insights to increase docking algorithms and grants an index of amino acid side chain flexibility, which potentiality aids in molecular docking. The atoms away from the binding site are neglected using ignored distant atom option. The active sites on the GSK-3β were analysed using Molegro Virtual Docker.

2.3. Molecular docking

Molegro virtual docker works on the basis of Moldock SE search algorithm The docking algorithm was set at a maximum iteration of 1500 with a simple evolution size of 50 and minimum of 5 runs. The population size was set at 50 with energy threshold of 100 at each step. The least minute was set as 10 minutes, the torsions/translations/rotations of the ligand protein interaction were tested and the one giving lower energies is chosen for further studies. The bonds flexibility of the ligands were fixed and the side chain flexibility of the amino acids in the binding cavity was set with a tolerance of 1.10 and strength of 0.90 for docking simulations. RMSD threshold for multiple cluster poses was set at <2.00.

The reranking score function is estimated more expensive than the scoring function used during the docking simulation but it is commonly better than the docking score function at analyzing the best pose among several poses originating from the same ligand (Thomson et. al., 2006). Binding affinities were estimated using Molegro data modeler The scoring function used by MolDock is derived from the piecewise linear potential (PLP) scoring functions which further improves these score with a new hydrogen bonding term and new charge schemes (Thomsen et. al., 2006).

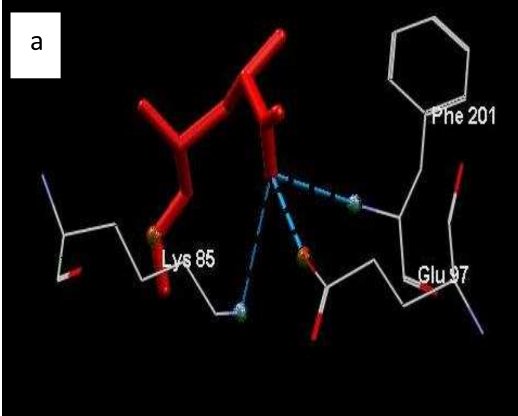
3. Result and Discussion:

Docking analysis was carried out for the selected phytoconstituents of *A.sativum* against the target protein GSK-3β using Molgro docking software so as to evaluate the efficacy of these compounds in suppressing the activity of target protein. The Moldock score, Docking score and rerank score of the potent flavonoids were displayed in table.1

Table-1: Moldock score for *A.sativum* against target protein

S. No.	Compound Name	Moldock Score	Rerank Score	Hydrogen Bond	Docking Score
1	Alliin	-70.0688	-53.4286	-6.4228	-69.742
2	Arginine	-67.7519	-60.2546	-6.4961	-74.0678
3	Allicin	-57.377	-47.8346	-0.5698	-61.151
4	L-Ascorbic Acid	-56.6499	-52.1574	-2.514	-59.0757
5	L-(+) Arabinose	-44.018	-45.4978	-4.5768	-50.5337

The phytoconstituent of *A.Sativum*Alliin showed more potent inhibitory activity against GSK-3β protein.Alliin is a naturally occurring plant bioflavonoid possesses a wide range of biological application including anticancer. The interaction of the compound with the target protein was analyzed using Molegro viewer module.



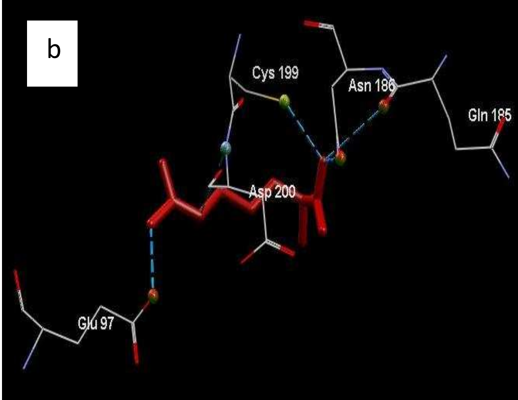


Figure.1 a) Interaction of Alliin with GSK-3β protein, b) Interaction ofArgininewith GSK-3βprotein visualized using Molegro viewer.

The receptor bound ligand Alliin bound deeply in binding pocket region. The active compound Alliin bound with receptor with a moldock score of -70.0688. The compound displayed interaction with seven different aminoacids with a total of 3 hydrogen bond interactions. Amino acid residue of GSK-3β involved in binding with phytoconstituents of *A.sativum* were displayed in Table 2.

Table-2: Amino acid residue of GSK-3β involved in binding with phytoconstituents of *A.sativum*

S. No.	Compound name	H2 bond interaction with Amino acid	No. of Hydrogen bond
1	Alliin	Lys 85, Glu 97, Phe 201	3
2	Arginine	Glu 96, Gln 185, Asn 186, Cys 199, Asp 200	5
3	Allicin	Cys 199, Asp 200	2
4	L-Ascorbic Acid	Lys 85, Asp 133	2
5	L-(+) Arabinose	Asp 133, Cys 199, Asp 200	4

4. Conclusion

The present molecular docking studies provide insights into inhibition of GSK-3β by phytoconstituents of *A.sativum*. Docking study propose that Alliin has a high binding affinity for GSK-3β protein. This study has led to the development of novel lead molecules which would help to develop enzymatic mechanisms which would facilitate in wound healing.

5. Reference

- Plyte, S., Hughes, K., Nikolakaki, E., Pulverer, B. and Woodgett, J. R. (1992)Biochim. Biophys.Acta 1114,147-162
- Parker, P. J., Caudwell, F. B. and Cohen, P. (1983) Eur. J. Biochem. 130, 227-234
- Wang, Q. M., Fiol, C. J., DePaoli-Roach, A. A., Roach, P. J. Glycogen synthase kinase-3 beta is a dual specificity kinase differentially regulated by tyrosine and serine/threonine phosphorylation. J. Biol. Chem. 269: 14566-14574, 1994.
- Reuter HD, Koch HP, Lawson LD. Therapeutic effects and applications of garlic and its preparations. In: Garlic. The Science and Therapeutic Application of Allium sativum L. and Related Species. Baltimore: Williams & Wilkins 1996;135:212
- Uchida Y, Takahashi T, Sato N. The characteristics of the antibacterial activity of garlic. Jpn. J. Antibiotics 1975; 638: 642-28.
- Thomsen R, Christensen MH. Mol Dock: A New Technique for High-Accuracy Molecular Docking. *J Med Chem.,* 2006, 49(11): 3315.

ANTIDIABETIC EFFECT OF ETHANOLIC ROOT EXTRACT OF *SALACIA RETICULATA* IN ALLOXAN INDUCED DIABETIC ALBINO RATS

J. Revathy
Assistant Professor, Bonsecours College for Women, Thanjavur
revathy.reva@gmail.com.

ABSTRACT

The antidiabetic effect of ethanolic extract of Salacia reticulata was determined. Various secondary metabolites were also identified and quantified by HPLC analysis. The animals were divided into four groups such as normal, diabetic untreated, diabetic treated with ethanolic extract and diabetic treated with reference drug Glibenclamide. The ethanolic extract of Salacia reticulata extract (200mg/kg body weight/rat/day) was given to the rats for 15 days orally. Blood samples were collected by retro orbital puncture and various biochemical parameters were measured using autoanalyser. The ethanolic extract shows significant reduction in blood glucose, glycosylated haemoglobin, blood urea, serum uric acid, serum creatinine, triglycerides, total cholesterol, phospholipids, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and increase in liver glycogen, insulin and lactate dehydrogenase (LDH). Our experimental findings with respect to the mechanism of action of extract in alloxan induced diabetic rats suggest that it enhances insulin secretion by the islets of langerhans, enhances peripheral glucose utilization and increases serum protein levels.

Key words: Thiobarbituric acid (TBA), Superoxide dismutase (SOD), Salacia reticulata, Alloxan, Glibenclamide, Glutathione (GSH), Catalase.

I. INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting the metabolism of carbohydrate, proteins, fat. It is a chronic disorder that prevents the body to utilize glucose completely or partially. It is characterized by raised glucose concentration in the blood [1].

It is due to the failure in the formation of insulin or liberation or action. Insulin is produce by the beta cells of islets of langerhans; any receding in the number of functioning cells will decrease the amount of insulin that can be synthesized. Carbohydrate is converted to monosaccharide glucose, which found in blood. Some carbohydrates are not converted. Plant drugs are frequently considered to less toxic and free from side effects than synthetic ones [2]. Medicinal herbs are significantly source of synthetic and herbal drugs. In the commercial market, medicinal herbs are used as raw drug. In traditional system of Indian medicine plant formulation and in several cases, combined extract of plants are used as the drug of choice rather than individual [3].

Salacia reticulata known as vairi or pitika in Sanskrit. It is a large woody climbing plant found throughout forest in India and Srilanka. In another language *Salacia reticulata* called as Ponkoranti (Tamil), Anukuducettu (Telugu), and Ekanayakam (Kannada) Koranti (Malayalam).

Salacia reticulata has been used an antidiabetic agent by ayurvedic practitioners. Extensive use of *Salacia reticulata* in ayurveda the roots and stem contains potent antidiabetic chemical constituents. There is currently no cure for diabetes. However, the condition can be managed well enough to allow most people to live normal lives. Treatment of diabetes focuses on two goals. The first is to keep blood glucose within a normal range and the second is to prevent complication from developing overtime [4].

The *Salacia reticulata* belongs to the Hippocrateaceae family. The root extract of *Salacia reticulata* have been reported to possess antidiabetic and antioxidant effects. The present study was designed to evaluate the antidiabetic activity of ethanolic root extract of the *Salacia reticulata* against alloxan induced diabetic albino rats. The effect of *Salacia reticulata* extract was compared to glibenclamide which is often used as a standard drug [5].

II MATERIALS AND METHODS

A. Collection of Plant Material

The plant material for the present investigation was collected from the field areas of Kumbakonam, Thanjavur District, Tamilnadu, India.

B. *Plant Extraction*

The powdered sample materials were extracted using 99% ethanol by cold percolation method. The powdered materials were soaked in ethanol (1:4) for 48 hours at 37°c. Then the filterate was filtered and distilled for recovering the solvent and then it was evaporated under reduced pressure at 50°c and the final content was used for the phytochemical work and for animal treatment [6].

C. Phytochemical Analysis

Phytochemical analysis was carried out qualitatively to identify the presence of various secondary metabolites such as alkaloids, flavanoids, tannins, phenols, steroids, glycosides, carbohydrates, aminoacids, proteins, saponins, terpenoids, ascorbic acid, coumarin, quinones and sulphur [7].

D. Animal Management

Wistar Albino rats (150-180gms) were selected for these studies. Six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment (obtained from Periyar pharmaceutical institute, Trichy).The animals were maintained in a control environment. They were provided with food and water adlibitum.

Source of chemicals

Alloxan chemical analytical grade was purchased from SD fine Chemicals Pvt. Ltd., Biosar. All other chemicals used, were obtained from Ranbaxy Research Laboratories, Glaxo Laboratories and Nice Pharmaceutical Company, India.

E. Induction of Hyperglycemia by Alloxan

Hyperglycemia was induced by intra peritoneal injection of freshly prepared aqueous solution of alloxan monohydrate (SD fine Chemicals Pvt. Ltd., Biosar) 150mg /kg, to overnight fasted rats. Control rats receive similar volume of vehicle, normal saline (2 ml/kg body weight) alone. Animals that did not develop hyperglycaemia after 48 hrs of alloxan injection were rejected and new animals were used. Immediately after confirmation of diabetes, rats were classified into four groups of five rats each.

F. Treatment Group Protocol

The animals were divided into four groups and each consists of five animals.

Group I - received normal saline and served as control.

Group II - treated with alloxan monohydrate 150 mg/kg served as diabetic control.

Group III - treated with ethanolic rhizome extract (200mg/kg)

Group IV- treated with glibenclamide (2.5mg/kg) and served as reference standard.

G. *Drug Administration*

Hyperglycemic rats were treated using ethanol extract of *Salacia reticulata* dissolved in Tween 40, through oral administration.

H. *Collection of Blood Sample*

At the end of the experimental periods, the rats were sacrificed. Plasma and serum were separated Treatment continued for 14 consecutive days. Before the treatment (0 day) and at the end of 7th and 14th day plasma levels were estimated using the glucose oxidase method. At the end of the experimental periods, the rats were sacrificed. Plasma and serum were separated from blood by centrifuging the samples at 5000 rpm for 10 min and stored in a refrigerator until analysed.

I. *Biochemical Analysis*

Blood samples were examined to determine plasma glucose[8] using radioimmunoassay kit and the serum concentrations of triglycerides (TG), high density lipoprotein (HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL), total cholesterol (TC), protein, urea were determined using commercial kits. The liver cytosolic contents of thiobarbituric acid reactive substance (TBA), Glutathione peroxide and ROS scavenging enzymes such as catalase and superoxide dismutase (SOD) were also determined using autoanalyser.

J. *Histopathological Studies*

On the 14th day, pancreatic tissues were taken from animals, which were fasted overnight, under ether anaesthesia. The whole pancreas from each animal was removed after killing the animals, was placed in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5μm thickness were cut and stained by haematoxylin and Eosin (H & E) for histological examination. The photomicrographs of histological studies are taken.

K *Statistical Analysis*

Results were statistically analysed by mean ± standard error. Significance between groups was estimated using student't' test [9].

III RESULTS AND DISCUSSION

Various phytochemicals such as alkaloids, flavanoids, tannins, phenols, steroids, glycosides, carbohydrates, aminoacids, proteins, saponins, terpenoids, ascorbic acid, coumarin, quinones, sulphur shows positive inference. They may help to prevent diseases like cancer and heart diseases besides their role to inhibit the microorganisms causing many diseases in human beings.

Flavanoids and other phenolic compounds have been reported as scavengers of free radicals and reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent the damage to lipids, proteins, enzymes, carbohydrates and DNA.

Table-1: Effect of ethanolic root extract of *Salacia reticulata* on serum glucose levels in alloxan induced diabetic albino rats.

S. No	Groups	Glucose (mg/dl)		
		Day 0	Day 7	Day 14
1.	Control (Normal Saline 2ml/kg)	90.3±4.6	90.8±3.6	90.8±6.6
2.	Diabetic Control (Alloxan 150 mg/kg).	263.3±18.9	262±19.2	262.3±17.7
3.	Diabetic + *Salacia retiuclata* (200mg/kg)	268±17.0	154.6±11.2*	118±9.7**
4.	Glibenclamide (2.5 mg/kg)	260±14.3	150±9.2*	101±6.2**

n = 6 Data are expressed as mean ± S.E
*p>0.01 Vs Control
**p>0.001Vs Control

Table-1 & Fig 1 reveals that the effect of ethanolic root extract of *Salacia reticulata* on the blood glucose level of experimental animals was determined at various days interval such as 0, 7 and 15 (fig.1). After oral administration of alloxan, there is a significant elevation in the plasma glucose levels by 2-3 times during experimental time periods when compared to normal groups. The administration of roots of *Salacia reticulata* extract in alloxan induced rats causes a significant reduction (p>0.01) in blood glucose level in diabetic rats at the days interval of 0, 7 and 14 respectively (Table-1). The administration of Glibenclamide alone shows a significant reduction in the level of blood glucose when compared to the treated group. This result indicates that 200 mg/kg of ethanolic root extract of *Salacia reticulata* have a very good hypoglycemic effect on alloxan induced albino rats and did not change the blood glucose level in normal rats.

The mechanism of hypoglycemic action probably involves direct or indirect stimulation of insulin secretion [10]. Alloxan includes "Chemical diabetes" in wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in decreases in endogenous insulin release. The chronic hyperglycemia of diabetes is associated with long term damage dysfunction and failure of various organs. In diabetic rats the utilization of impaired carbohydrate leads to accelerate lipolysis, resulted in hyperlipidemia. Ethanolic extract of roots of Salacia reticulata exhibited a significant antihyperglycemic effect in normal and alloxan induced diabetic rats. However the recent study suggests that the ethanolic extract of roots of *Salacia reticulata* exhibited action similar to glibenclamide (reference hypoglycemic drug). That is stimulation of the surviving β-cells to release more insulin, which support our hypothesis.

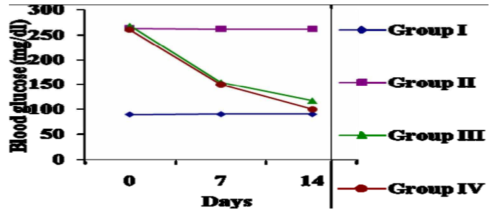


Figure 1: Effect of ethanolic root extract of *Salacia reticulata* on plasma glucose level

Table-2: Effect of ethanolic root extract of Salacia *reticulata* on serum lipid profile in alloxan induced diabetic albino rats

S. No.	Groups	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Cholesterol mg/dl
1.	Normal	76.12± 4.12	25.60± 1.83	15.22 ±0.82	39.12± 4.13	78.84± 6.27
2.	Diabetic Control (Alloxan 150 mg/kg)	114.22± 7.43	18.0± 2.6	22.8± 1.48	145.41 ±1.2	242± 7.38
3.	Diabetic + *Salacia reticulata* (200 mg/kg)	86.54* ± 7.1	24.93* ± 6.52	17.30* ±1.42	31.1** ±0.4	86.0± 4.32
4.	Glibenclamide (2.5 mg/kg)	83.74*± 4.9	33.82* ± 6.7	16.74* ±0.98	29.8** ± 1.2	80.94 ±4.98

n = 6 Values are expressed as mean ± S.E
***p<0.01 Vs Control**
****p< 0.001 Vs Control**

Table-2 shows that the effect of ethanolic root extract of Salacia reticulata on triglyceride, total cholesterol, HDL, LDL and VLDL in normal and diabetic rats. The result showed that the level of triglyceride, cholesterol, HDL, LDL and VLDL have increased in alloxan induced rats when compared to the normal rats. The administration of roots of *Salacia reticulata* (200mg/kg) and Glibenclamide (2.5 mg/kg) significantly decreased (p<0.01) the level of triglyceride, cholesterol, LDL, HDL and VLDL when compared with control diabetic rats.

Hypercholestremia and hypertriglyceridemia have been reported to occur in alloxan induced diabetic rats and a significant increases observed in the present experiment was in accordance to these studies ([11], [12]). Under normal circumstances, insulin activates enzymes lipoprotein lipase and hydrolyses triglycerides. However in insulin defect subject, it fails to active the enzymes and causes hyper triglyceridemia in the present investigation, ethanolic extract of roots of *Salacia reticulata* and Glibenclamide acted in a similar way by lowering the triglyceride levels by activation of lipoprotein lipase. In addition, treatment of rats which ethanolic extract of roots of *Salacia reticulata* markedly decreased the level of total cholesterol. These results indicated that the roots of *Salacia reticulata* had a beneficial effect on the hyperlipidemia associated with hyperglycemia.

Table-3: Effect of ethanolic root extract of *Salacia reticulata* on Protein and Urea (mg/dl) in diabetic induced albino rats

S. No.	Groups	Protein (mg/dl)	Urea (mg/dl)
1.	Normal	5.1± 0.43	22 ±0.23
2.	Diabetic Control (Alloxan 150 mg/kg)	1.91±0.06	34± 0.47
3.	Diabetic + *Salacia reticulata* (100 mg/kg)	3.6 ±0.13	24.6 ±1.7
4.	Glibenclamide (2.5 mg/kg)	4.1* ±0.22	22.8* ±1.1

n = 6, Values are expressed as Mean ±S.E
***p<0.01 Vs Control**
****p< 0.001 Vs Control**

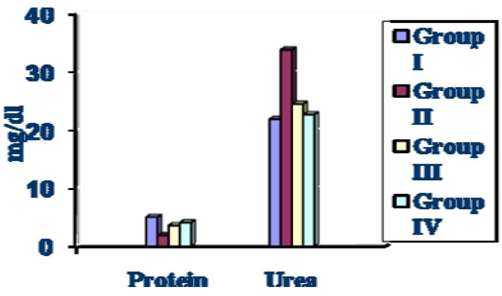


Figure 2: Effect of ethanolic root extract of *Salacia reticulata* on Protein and Urea in diabetic induced albino rats.

Table-3 & Fig 2 reveals that the effect of ethanolic root extracts of *Salacia reticulata* on protein and urea level in normal and diabetic rats. The results showed that decreased protein level and increased urea level in diabetic rats when compared with normal rats. The administration of roots of *Salacia reticulata* extract (200 mg/kg) in alloxan induced rat shows a significant reduction (p<0.01) in urea level and increase in protein level.

Protein levels are not completely elucidated, a number of evidences accumulated from the various biochemical studies are how suggesting that the protein Tyrosine phosphatase is a major negative regulator of insulin receptor signaling. Accordingly protein tyrosine phosphatase is considered as a attractive target for the treatment of type-2 diabetes and related metabolic syndromes. In the present study, elevated level of total protein have been found out in alloxan include diabetic rat.

The results coincide with the results of which work on antidiabetic effect of *Pterocorpus marsupium* in alloxan induced diabetic rats [11]. Treatment with ethanolic extract of roots of *Salacia reticulata* significantly increased the total protein and urea level is decreased in alloxan induced rats which infers the protective effects of the plant extract against alloxan induced pancreatic damage

Table-4: Effect of ethanolic root extract of Salacia reticulata on Catalase and Superoxide dismutase activity in alloxan induced diabetic albino rats

S. No.	Treatment	Catalase (mg/liver protein)	Superoxide dismutase (mg/liver protein)
1.	Normal	297.07±18.1	77.91 ±6.21
2.	Diabetic Control (Alloxan 150 mg/kg)	165.75± 6.78	32.31± 0.67
3.	Diabetic + *Salaciareticulata* (200 mg/kg)	386.27± 7.54*	68.55 ±4.65*
4.	Glibenclamide (2.5 mg/kg)	380.02 ±5.34	60.1± 3.25

n=6, Datas are expressed mean ± S.E
***p<0.01 Vs Control**
****p< 0.001 Vs Control**

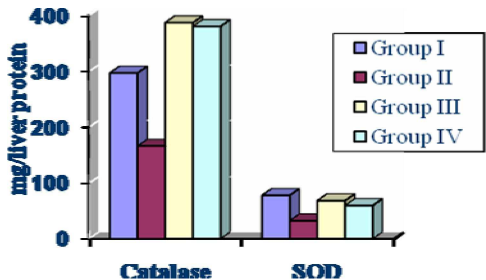


Figure 3: Effect of ethanolic root extract of *Salacia reticulata* on Catalase and SOD enzymes in alloxan induced diabetic albino rats

Table-4 & Fig 3 shows the intraperitoneal injection of alloxan to the rat causes significant decreases in the level of catalase and superoxide dismutase. After the treatment with 200 mg/kg of roots of *Salacia retiuclata*, there is a significant increase in the level of catalase and superoxide dismutase when compared to diabetic control. Thus this result indicates that the ethanolic root extract of *Salacia reticulata* lightly increases in the enzymes such as catalase and superoxide dismutase.

Table-5: Effect of ethanolic root extract of *Salacia reticulate* on Glutathione Peroxide and TBA in alloxan induced diabetic albino rats

S. No.	Treatment	Glutathione peroxide (mg/liver protein)	TBA (mg/liver protein)
1.	Normal	0.961± 0.030	1.27 ±0.389
2.	Diabetic control (Alloxan)	0.747± 0.053	1.79± 0.14
3.	Diabetic + *Salacia reticulata* (200 mg/liver protein)	0.874± 0.074*	1.77± 0.12*
4.	Glibenclamide (2.5 mg/kg)	0.810 ±0.052	1.25 ±0.08

n=6, Data are expressed mean ± S.E
***p<0.01 Vs Control**
****p< 0.001 Vs Control**

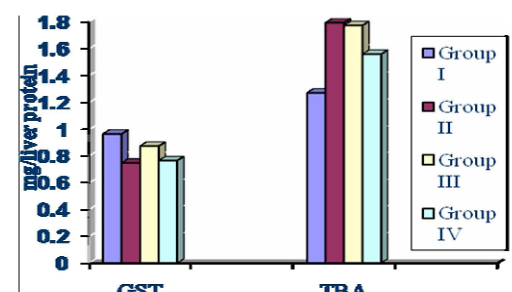


Figure 4: Effect of ethanolic root extracts of *Salacia reticulata* on Glutathione peroxide and TBA in alloxan induced diabetic rats

Table-5 & Fig 5 Intraperitoneal injection of alloxan to the rats causes slight decreases in a level of glutathione peroxidase and slight increases in the level of TBA respectively. After the treatment with 200 mg/kg of Salacia retiuclata root extract, there is a slight reduction in the level of TBA and slight increases in the level of glutathione peroxidase.

Enzymatic antioxidant such as SOD and catalase are considered primary enzyme since they are involved in the direct elimination of ROS[13]. SOD is an important defense enzyme

and scavenging O2- anion from hydrogen peroxide and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reaction [14]. The antioxidant enzyme such as SOD, catalase, glutathione peroxide and TBA are known to be inhibited in diabetes mellitus, as a result of non-enzymatic glycosylation and oxidation [15]. In the present study, the activities of SOD, catalase, glutathione peroxide and TBA decreased in diabetic rats as reported earlier which could be due to inactivation caused by alloxan generated ROS([15], [16]).

A. Effect on Pancreas

Alloxan induces extensive damage to the β cells of islets of langerhans. Restoration of normal cellular population and size of islets with hyperplasia were seen in extract treated groups. The partial restoration of normal cellular population and enlarged size of β cells with hyperplasia were indicative of the antidiabetic potential of the plant.

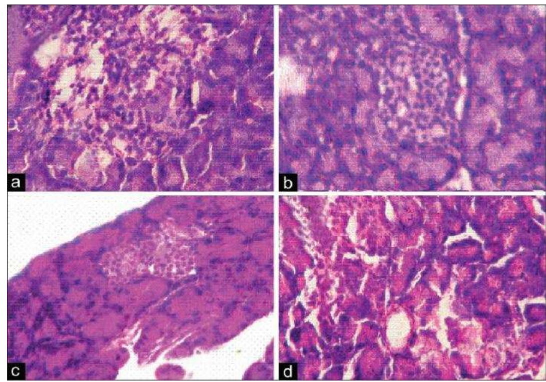


Figure 6: Effect of *Salacia reticulata* on histology of pancreas. Figure illustrates the photomicrographs of pancreas (haematoxylin and eosin staining) of untreated rats (a), alloxan treated rats (b), Salacia reticulata (200 mg/kg) treated rats (c), and Glibenclamide(2.5mg/kg) treated rats (d). Microscope magnification (×100)

IV. CONCLUSION

Alternative strategies to the current modern pharmacotherapies of diabetes mellitus are urgently needed because of the inability of existing modern therapies to control all the pathological aspects of the disorders, as well as enormous cost and poor availability of the modern therapies for many rural populations in developing countries. The present study investigated both qualitative and quantitative analysis of ethanolic root extract of *Salacia reticulata.* The roots of *Salacia reticulata* produced a significant reduction in blood glucose level in alloxan induced diabetic albino rats and comparable with that of the standard drug glibenclamide. The treatment with the root of *Salacia reticulata* causes a significant reduction in the level of triglycerides, LDL, VLDL and cholesterol and increase in HDL in the alloxan induced diabetic rats as compared with the diabetic group. This results indicate that the ethanolic extract of (200 mg/kg) of root of *Salacia reticulata* prevent hyperlipidemic and its related disorder. The evaluation of antioxidant enzyme such as catalase, SOD, glutathione peroxide and TBA decreased in their activity was observed in alloxan treated rats. The treatment with ethanolic root extract of *Salacia reticulata* shows a significant increase in protein level and decreased in urea in the alloxan treated rats. This study confirmed that the root of *Salacia reticulata* possesses various phytoconstituents, which may contribute its hypoglycemic and hypolipidemic effect. This study paved a way for future investigators to analyze and to isolate a novel pharmacologically active compound from root of *Salacia reticulata* which is used to treat metabolic disorder.

REFERENCES

1. Robinson, K., Sathish kumar, T. and Srinivas, S., (1986), Medicinal plants for treatment of diabetes mellitus, *Indian Journal of Clinical Biochemistry,*52 (56-60).
2. Pari, L. and Saravanan, G., (2002). Antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetes mellitus. *Comparative Biochemistry and Physiology Part* C 131: (19-25).
3. Nair, N., Kalariya, T. and Sumitra Chanda, (2005). Antibacterial activity of some selected Indian medical flora. *Turkee Journal for Biology*, (41-47).
4. Alexandria, P. (2003). Antidiabetic and antihyperlipidemic effect of neem seed, kernel powder on alloxan diabetic rabbits. *Indian Journal of Pharmacology*, 29: (162-167).

5. Illavarasan, R., Jayachandran, T., Deecaraman, M., Aravindan, P., Padmanaban, N. and Krishnan, M.R., (2008). Antidiabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin induced diabetic rats. *Journal of Medicinal Plants Research* Vol. 2(9): (246-249).
6. Helder C. Oliveira, Maisa P. dos santos, Rafael Grigulo, Layala L. Lima, Domingos T.O. Martins, Joaquim C.S. Lima, Luiz F. Stoppiglia, Carbene F. Lopes and Nair H. Kawashita,(2008). Antidiabetic activity of *Vatairea macrocarpa* extract in rats. *Journal of Ethanopharmacology* 115 : (515-519).
7. Gopiesh Khanna, V., and Kannabiran, K., Larvicidal effect of *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostrata* against culex qinquifaciatus mosquito larvae. *African Journal of Biotechnology* Vol.6 (3) pp 307-311, (2006).
8. Trinder, P. Determination of glucose in blood using oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.*, 6, 24-27, (1969).
9. Snedeeor G.W., and Cochran W.G., in "Statistical Methods", Oxford and IBH Publishing Co., New Delhi, 33 (1997).
10. Carson, J.F.(1987). Chemistry and Biological properties of Garlic, Food rev. Internet. 3, (71-103).
11. Dhanapal, S.P., Kokate, C.K., Ramanathan, M., Kumar, E.P. and Suresh, B.(2006). Hypoglycemic activity of *Pterocarpus marsupium* Roxb. *Phytotherapy research*; 20(1). (4-8).
12. Sharma, S.R., Dwivedi, S.k. and Swarup, D., (1997). *Journal of Ethanopharmacology,* 58 (39-44).
13. Arulselvan, P. and Subramanian S.P., (2007). Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic β-cells in experimental diabetes in rats. *Chemica-Biological Interactions,* 165 (155-164).
14. Manonmani, G., Bhavapriya, V., Kalpana, S., Govindaswamy, S. and Apparanantham, T., (2005). Antioxidant activity of *Cassia fistula* (Linn.) flowers in alloxan induced diabetic rats. *Journal of Ethanopharmacology*, 97 (39-42).
15. Al-Azzawie, H. and Alhamdani, M.S.S., (2006). Hypoglycemic and antioxidant effect of oleuropein in alloxan diabetic rabbits. Life Sciences 78, (1371-1377).
16. Sepici-Dincel, A., Sengelen, M. and Yesilada, E., (2007). Effects of in vivo antioxidant enzyme activities of myrtle oil in normoglycaemic and alloxan diabetic rabbits. *Journal of Ethanopharmacology*, 110 (498-503).

REVIEW ON NANOPARTICLE- AN INNOVATIVE DEVELOPMENT IN CANCER THERAPY

Sajel. S*, Praveen. D & Ranadheer Chowdary.P
School of Pharmaceutical Sciences, Vels University.

ABSTRACT

Cancer remains a significant crises leading to the amplified effects of morbidity and mortality. Treating cancer has become one of the most vital roles for healing patients. Cancer therapy although not new, it is a rapid progressing and being used in all way for targeting the affected cells, they have indefinite bio distribution with deprived bioavailability with low therapeutic index and low safety index. The nanoparticles are being designed in a finest size and character such that it overcomes the time essential for the cancer drugs to circulate in the blood stream, since nanoparticles carry the active ingredient they are intended to target the pathophysiology of distinct tumours. Drug resistance is another barrier that blocks the efficacy of cancer chemotherapeutic agent. Nanoparticles has a unique mechanism of accumulating inside the cell hiding from P-glycoprotein, a notable mediator responsible for multi drug resistance effecting increased intra cellular concentration of the drug. Hence recent development of nanoparticle is an upcoming challenge drug delivery system in the field of oncology.

Key points: *Nanoparticle, Tumour cells, Drug delivery, Resistance*

Introduction

Cancer plays a promising role for clinicians for treating varied population. The recent advances in cancer have shown a spot light in healing cancer at molecular level. The inhibition of cancer cells is based on the selection of the specific pathway for the eradication of the disease., but when there is an alteration in the targeting pathway the cells do not recognise the chemotherapy and the cancer cells continue to grow as tumour inside the body[1].

Nanotechnology – the man made device of which the device itself or the specific components be 1-1,100 nm rage atleast in one dimension will allow reduction of screening tool for cancer diagnosis and treatment as a single device thereby making it a cost efficient [2].There are several nanoparticle drug delivery systems being available all over the country.

This review on harmony with basic concepts of nanotechnology defines the features of nanoparticle its target and efficacy and other crucial unsolved trouble faced in cancer and improvement of patient survival[4].

Growth of tumours

Cancer cell a very minute micro size cell if surrounded by a healthy tissues they have an ability to imitate the cells at higher rate damaging the nutrient supply and metabolic waste products. This helps in formation of tumour colony that affects the healthy cells which is unable to defeat the cancer cells due to lack of nutrients from the blood stream. Now the healthy cells will be replaced by cancer cells until it reaches the diffusion limited maximum size ($2mm^3$)[5]. The chain reaction in continuous division of cancer cells occur because the tumour cells require basic necessity what the normal cells require (oxygen, glucose, amino acids)if there is lack of this essential building block the cancer cell do not trigger apoptosis and continue replicating until new blood vessels are formed[5]. the proliferation rate will be equal to the rate of cell death at a steady state. It is said that it takes years to get proliferated all over the body until the cancer cell reaches the circulation with same diffusion limit maximum size[6].

Nanoparticles play a key role in targeting and preventing the tumour progression.

Nanoparticle in general

Nanoparticle as the name suggests they are smaller particles constructed to carry a therapeutic effect which are loaded with proteins that act as a key to enter the tumour cell[8].

Nanoparticle has several roles to play in oncology. They serve as contrast agents that are developed for detecting the tumour cell. There are various categories for detecting the cancer which are diagnosed by Magnetic Resonance Imaging especially Super Paramagnetic nanoparticle are used for MRI.

Use of Nano scale crystals as a Quantum dots, a stable light emitters by the use of cadmium selenide, a semiconductor can be linked to the antibodies and can be combined to measure assays used for multiple diagnosis. Quantum Dots are very effective in detecting the tumour markers[9].

Nanoparticle is being used as a biomarker for tumours. Biomarkers help in accurate sensitivity of cancer diagnosis by preventing the disintegration of tumour markers[9].

Nanoparticle in cancer chemotherapy has marked its position as a drug delivery vehicle with high therapeutic effect. They act as delivering vehicle of drugs loaded with active components and help in targeting the malignant cells sparing the healthy tissues[8].

Nanoparticle and its drug delivery platforms

As drug delivery targets Nanoparticle has various forms such as polymers, lipids, viral nanoparticles and carbon nanotubes which are being discussed below.

Polymers: there are polymers in nanoparticles such as polymeric nanoparticle, dendrimers. The polymers are being prepared based on the drug. The drug is either physically entrapped or covalently bound, thereby forming a capsule structure (polymeric nanoparticle) or hyper branched macromolecule (dendrimers) or core / shell (polymeric micelles) based on this two examples are being discussed below.

Polymeric nanoparticles: They are natural polymers containing either of this albumin, chitosin, and heparin. The natural polymer is designed for the delivery of drugs, DNA, proteins, oligonucleotides. In this nanoparticle the drugs are conjugated to the side chain of linear polymer with cleavable bond (linker) [6].

EXAMPLE: Albumin Taxol[12], PGA Taxol[11], HPMA Dox (PK_1)[14]

Polymeric Micelles: They are Nano sized shell/core structure in aqueous media. They are amphiphilic block copolymers with core - hydrophilic and shell- hydrophilic structure. The core serve as a drug delivery for hydrophobic drugs and the shell serve as a balance where the polymers are rendered water soluble making suitable for I.V administration.

EXAMPLE: PEG-plunoric-DOX[16], PEG-PAA-DOX (NK911)[17], PEG-PLA-Taxol (Genexol-PM) [18].

Dendrimers: dendrimers are synthetic nanoparticle with 10nm size. They are designed with functionalizable terminal group branching out from central core, by conjugation to functional end group or either through encapsulation of central activity, leading to controlled release of therapeutic agents. The conjugation of several molecules is easy if the characters of dendrimers are simply alterable[6].

EXAMPLE: PAMAM-MTX[21], PAMAM Platinate[18].

Liposomes: they are archetypal, simplest form of nanovector, though liposomal drug delivery nanoparticle is an old concept but it plays a major role with various encapsulated formulation. They are self assembling closed colloidal structure compiled with lipid bilayers since being old several cancer drugs have been established with this drug delivery concept[6].

EXAMPLE: Liposomal Danurobucin[22], Pegylyted Liposomal DOX (Doxil) [23].

Viral naopartiles and carbon nanotubes: Several viruses are designed and introduced in a nanotechnology application for tissue targeting and drug delivery, they are mutivalent self assesment structures along with protein cages[6].

EXAMPLE:HSP-DOX[27], CPMV-DOX[29]

Carbon nanotubes are cyinder with carbon compiled of benzene ring. They are used as an diagnostic devices for detecting the DNA, proteins[6].

EXAMPLE: CNT-MTX[34], CNT amphotericin[33].

Targeting cancer cell

The key role of treating the carcinogen with anticancer drug is to reach the specific cancer tissue by penetrating the barriers without affecting the normal body function. The cancer

drugs sholuld have the capacity to kill the tumour cells sparing the other healthy cells.For diminishing the mortality and morbidity of the cancer patients and to improve the quality of life, the intracellular drug concentration and dose limiting toxicities should be focused simutaneously[6]. These two stratergies are present in nanoparticle drug delivery system. The injected nanoparticle are controlled by their size and surface charecters. They have the capability to cirulate in the blood stream for a longer time interval[17]. This is because they escape from the macrophages capturing which are stored in retinoendothelial system such as livers and spleen and hence reaches targetted tumour tissues. There is a specific tumour vessel pathway that accumulates macromolecules and nanoparticles with molecular weight above 50kDa. This recruitment of new vessel near the tumour mass is due to supply of nutrients required for the cancer cell growth. All the drug delivery system works by binary conjugation by making use of passive targeting mechanism[32]. The recent development and exposure to wide range of liposomes and polymers as vehicle carrying drug molecule hikes up the number of drugs to be conjugated for the targeting the cancer molecule. Still various clinical studies are being done under this selection of drug moieties and making them more efficient for cancer cell target.

Nanoparticle a choice to overcome Resistance

Drug resistance is a crucial barrier where every drug fails to reach their target intended for the action or preventing the disease. Drug resistance usually lowers the therapeutic efficacy of cancer drugs . There are various mecahnism leading to drug resistance of a drug. Among them P-Glycoprotein is commonly and widely examined. Nanoparticle have the ability to overcome the P-Glycoprotein Resistance. The nanoparticle hides itself from the recognition by P-glycoprotein efflux pump by covering themselves in an endosome while entering the cell which leads to increased intracellular drug concentration. Likewise every drug delivering platform of nanoparticle influence the inhibition of drug resistance in several pathways[6,9,10].

Nanoparticle pros and cons

Nanoparticle has a multifunctional platforms that act in cancer biology as materials for targeting the diagnosis and imaging of cancer and also as a vehicle for the delivering cancer drugs. The size and structure of nanoparticle has a positive role in controlled release of drug and targetting the cancer.The nanoparticle proves to be a value with better therapeutic effect since they are devoid of resistance.

Though nanoparticle serve as an advantage for the cancer drug target they also suffer certain disadvantages .

Since nanoparticles are less than 200nm size they are no longer been absorbed by the phagocytes(digestive enzyme) hence they move freely. Certain non bio degradable nanomedicines are accumulated in certain organs like liver. There are ceratin immune impairments which are still under newer clinical study.

Future concerns regarding Nanomedicine

Nanomedicine has the ability to make the positive impact to people from all phases of life. Nanotechnology will radically change the way of diagnosis , treatment and prevention of cancer. Nanotechnology can increase the lifespan of the human being by improving the efficiency and quality. Hence nanoparticle is the miracle fruit of curing complicated cancer and thereby improving the treatment.

REFERENCES

1. Langer, R. Drug delivery and targeting. *Nature* **392**, 5–10 (1998).
2. Duncan, R. The dawning era of polymer therapeutics. *Nature Rev. Drug Discov.* **2**, 347–360 (2003).The definitive, state-of-the-art review of polymer technology for drug delivery application. This paper is so exhaustive that we chose not to focus on polymer nanotechnology in our review.
1. Li, K. C. P., Pandit, S. D., Guccione, S., Bednarski, M. D. Molecular imaging applications in nanomedicine. *Biomed. Microdevices* **6**, 113–116 (2004).

2. Allen, T. M. Ligand-targeted therapeutics in anticancer therapy. *Nature Rev. Drug Discov.* **2**, 750–763 (2002).
3. Jain, R. K. The next frontier of molecular medicine: delivery of therapeutics. *Nature Med.* **4**, 655–657 (1998).
4. Srinivas, P. R., Barker, P. & Srivastava, S. Nanotechnology
5. in early detection of cancer. *Lab. Invest.* **82**, 657–662 (2002).
6. Ross JS, Schenkein DP, Pietrusko R, et al. Targeted therapies for cancer 2004. Am J Clin Pathol 2004; 122:598 ^ 609.
7. Morgillo F, Lee HY. Resistance to epidermal growth factor receptor-targeted therapy. Drug Resist Updat 2005;8:298^310.
8. Maeda H. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzyme Regulation 2001; 41:189^207.
9. Allen TM. Ligand-targeted therapeutics in anticancer therapy. Nat Rev Cancer 2002; 2:750^63.
10. Larsen AK, Escargueil AE, Skladanowski A. Resistance mechanisms associated with altered intracellular distribution of anticancer agents. Pharmacol Ther 2000; 85:217^ 29.
11. Rawat M, Singh D, Saraf S, Saraf S. Nanocarriers: promising vehicle for bioactive drugs. Biol Pharm Bull 2006; 29:1790^ 8.
12. GradisharWJ, Tjulandin S, Davidson N, et al. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J Clin Oncology 2005; 23: 7794^803.
13. Green MR, Manikhas GM, Orlov S, et al. Abraxane, a novel Cremophor-free, albumin-bound particle form of paclitaxel for the treatment of advanced nonsmall- cell lung cancer. Ann Oncology 2006; 17:1263^ 8.
14. Nyman DW, Campbell KJ, Hersh E, et al. Phase I and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced non hematologic malignancies. J Clin Oncology 2005;23:7785^93.
15. Li C. Poly(L-glutamic acid)-anticancer drug conjugates. Adv Drug Deliver Revering 2002;54:695^713.
16. Sabbatini P, Aghajanian C, Dizon D, et al. Phase II study of CT-2103 in patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma. JClin Oncology 2004;22:4523^31.
17. Bhatt R, de Vries P,Tulinsky J, et al. Synthesis and in vivo antitumor activity of poly(l-glutamic acid) conjugates of 20S-camptothecin. JMed Chem 2003; 46: 190 ^ 3.
18. Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discovery 2003; 2:347^60.
19. Vasey PA, Kaye SB,Morrison R, et al. Phase I clinical and pharmacokinetic study of PK1 [N-(2-hydroxypropyl) methacrylamidecopolymerdoxorubicin]: firstmember of a new class of chemotherapeutic agents-drugpolymer conjugates. ClinCancerRes1999;5:83^94.
20. Adams ML, Lavasanifar A, Kwon GS. Amphiphilic block copolymers for drug delivery. J Pharm Sci 2003; 92:1343^55.
21. Batrakova EV, Dorodnych TY, Klinskii EY, et al. Anthracycline antibiotics non covalently incorporated into the block copolymer micelles: in vivo evaluation of anti-cancer activity. BrJ Cancer 1996; 74: 1545^ 52.
22. Nakanishi T, Fukushima S, Okamoto K, et al. Development of the polymer micelle carrier system for doxorubicin. JControl Release 2001; 74: 295^302.
23. KimTY, Kim DW, Chung JY, et al. Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. Clin Cancer Res 2004; 10:3708 ^ 16.
24. Nasongkla N, Bey E, Ren J, Ai H, Khemtong C, Guthi JS. Multifunctional polymeric micelles as cancer- targeted, MRI-ultrasensitive drug delivery systems. Nano Letter 2006; 6: 2427^30.
25. Svenson S, Tomalia DA. Dendrimers in biomedical applications Ireflections on the field. Adv Drug Deliver Rev 2005; 57:2106^ 29.

26. Malik N, Evagorou EG, Duncan R. Dendrimerplatinate: a novel approach to cancer chemotherapy. Anticancer Drugs 1999; 10: 767^76.
27. Markman M. Pegylated liposomal doxorubicin in the treatment of cancers of the breast and ovary. Expert Opin Pharmacotherapy 2006; 7:1469^74.
28. Rivera E. Current status of liposomal anthracycline therapy in metastatic breast cancer. Clinical Breast Cancer 2003; 4: S76^83.
29. Rosenthal E, Poizot-Martin I, Saint-Marc T, Spano JP, Cacoub P, Group DNXS. Phase IV study of liposomal daunorubicin (Dauno Xome) in AIDS-related Kaposi sarcoma. AmJClin Oncology 2002; 25: 57^ 9.
30. Hofheinz RD, Gnad-Vogt SU, Beyer U, Hochhaus A. Liposomal encapsulated anti-cancer drugs. Anticancer Drugs 2005; 16:691^707.
31. 32.Wu J, Liu Q, Lee RJ. A folate receptor-targeted liposomal formulation for paclitaxel. Int J Pharm 2006; 316:148^53.
32. Manchester M, Singh P. Virus-based nanoparticles (Vnanoparticles): platform technologies for diagnostic imaging. Adv Drug Delivery Rev 2006;58: 1505^ 22.
33. Singh P, Destito G, Schneemann A, Manchester M. Canine parvovirus-like particles, a novel nanomaterial for tumor targeting. J Nanobiotechnology 2006; 4:2.
34. Flenniken ML,Willits DA, Harmsen AL, et al.Melanoma and lymphocyte cell-specific targeting incorporated into a heat shock protein cage architecture. Chem Biology 2006; 13:161^ 70.
35. Flenniken ML, Liep old LO, Crowley BE,Willits DA, Young MJ, Douglas T. Selective attachment and release of a chemotherapeutic agent from the interior of a protein cage architecture. Chem Commun (Camb) 2005; 4: 447^9.
36. Bianco A, Kostarelos K, Partidos CD, Prato M. Biomedical applications of functionalised carbon nanotubes. Chem Community 2005; 5: 571^7.
37. Bianco A, Kostarelos K, Prato M. Applications of carbon nanotubes in drug delivery. Curr Opin Chem Biology 2005; 9: 674^9.
38. Wu W, Wieckowski S, Pastorin G, et al. Targeted delivery of amphotericin B to cells by using functionalized carbon nanotubes. Angew Chem Int Ed England 2005; 44:6358^62.
39. Pastorin G, Wu W, Wieckowski S, et al. Double functionalisation of carbon nanotubes for multimodal drug delivery. Chem Community 2006; 11: 1182^4.

KINETICS OF BATCH ADSORPTION OF IRON(II) IONS FROM AQUEOUS SOLUTION USING ACTIVATED CARBON FROM CORCHORUS OLITORIUS - L - LEAVES

S. Subasri[1*], S. Arivoli[2*], V. Marimuthu[2] & N. Mani[1]

[1]Department of Chemistry A.V.V.M Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India.*

[2] Department of Chemistry, Thiru.Vi.Ka. Government Arts College Thiruvarur, Tamil Nadu, India.*

[*]Authors for Correspondence : arivu3636@yahoo.com, srisuba123@gmail.com

ABSTRACT

Corchorus Olitorius - L- Leaves obtained from nearby Thiruvarur district, having the particle size (53–150 µm) was used as an adsorbent for the removal of Fe (II) ion from aqueous solution. The effect of various factors (temperature, adsorbent dose and Initial pH) on adsorption of Fe (II) on Corchorus Olitorius - L- Leaves was investigated. The effect of pH shows that the amount adsorbed increased with the increase of pH of solution. The equilibrium adsorption isotherms were analyzed by Langmuir and Freundlich equations. Both Langmuir and Freundlich models can describe the adsorption equilibrium but the Langmuir model shows better agreement. The amount adsorbed increased with the increase of temperature suggests the formation of dimer in the contact region. SEM micrographs and differential molar isosteric heat of adsorption (ΔH) calculated at different surface coverage, indicate that the surface is heterogeneous having energetically different adsorption sites. Values of n calculated from Freundlich plots indicate that adsorption of Fe(II) on Corchorus Olitorius - L- Leaves is spontaneous. At high surface coverage, the differential heat of adsorption versus surface coverage plot shows maximum value indicating the occurrence of structural rearrangement in the adsorbate. With the increase of adsorbent dose, amount adsorbed increased due to the increased surface area of adsorbent. It being rich in Medicinal uses.

Keywords: *Iron, Activated Corchorus Olitorius - L - Leaves Nano Carbon (ACONC), Adsorption models, Equilibrium.*

1. INTRODUCTION

Water is the main component for living organisms, and the increase in water pollution as a result of progress in the industrial technologies, has been reduced using many methods to treat the wastewater [1]. The choice of suitable methods is controlled by different factors such as the efficiency of removing the pollutant materials, the availability of the used chemicals and the chemistry of the contaminated materials beside the process cost [2]. It is well–known that the pollutants in wastewater discharge from industrial effluents, sewage, sludge, pesticides, and fertilizers. The composition of the contaminated water depends on the source of the pollutant, the chemical composition of the original water whether it is surface or underground water and then the chemical reaction with the soil. For example, groundwater contains one or more contaminants like iron, manganese, ammonium, methane and natural organic matter, e.g., humic acid. Hence, before using this water supply for agro-irrigation purposes, these contaminants should be removed or reduced to the acceptable levels. Iron and manganese, which are usually present in the groundwater as divalent cations, considered to be contaminants mainly due to their organoleptic properties. The maximum recommended levels of Fe in drinking water are 0.3 mg/L, respectively. There are various methods for removing Fe(II) cations from the wastewater including ion-exchange method [3], oxidation by oxidizing agents such as chlorine and potassium permanganate [4], activated carbon and/or other filtering materials [5-7], supercritical fluid extraction (Andersen and Bruno, 2003), bioremediation [8], and treatment with limestone [9]. Some of these methods are simple and economic while the others are complicated and expensive. In oxidation treatment, oxygen, chlorine or potassium permanganate ($KMnO_4$), is generally used for Fe(II) oxidation. Adsorption using activated carbon is an effective technique to remove heavy metals from wastewater [10] that is due to that activated carbon has a pore size distribution which controls its adsorption capacity, a chemical structure that influences its interaction with polar and non-polar adsorbate, and active sites which determine the type of chemical reactions with other molecules [11].

However, in developing countries such as India, traditional activated carbon remains an expensive material for heavy metal removal. Recently, many researchers have been published

in the literature including preparation of activated carbons from various cheaper and alternative materials, e.g., agricultural by-products and biomass materials, using chemical activation with H_3PO_4 [12-14]. However, there is only limited research on the preparation of activated carbons from woody biomass such as sawdust for uptaking heavy metals such as Hg (II) from wastewaters [15,16] reported that the removal of Fe, Mn and Cu ions from acid mine drainage (AMD) by precipitation with NaOH depends on the pH value besides the oxidation state of the removed cations. On the other hand, various authors found that the removal of iron and other heavy metals by activated carbon depends on the nature of carbon (porosity, surface area, oxygen functional groups, etc.). Within the frame of this policy, the present paper narrates the investigation of Fe(II) ions removal from aqueous solution using adsorption methods in order to determine the optimum pH for the effective removal. The adsorption was carried out using an activated carbon obtained from Corchorus Olitorius-L-Leaves. The adsorption data was analyzed by using Langmuir and Freundlich isotherm models. Overall, this study was also intended to determine the efficiency and the optimum conditions in adsorption processes for removal of iron cations.

Corchorus olitorius leaves is an edible leafy vegetable that is a member of the genus Corchorus, classified under the subfamily Grewioideae of the family Malvaceae. It is widely found in tropical and subtropical areas from Asia to Africa valued as food and for its strong fiber. Corchorus olitorius leaves has long been used as food staple since ancient times by Jewish people and Egyptians hence derived its English names Jew's mallow and Egyptian spinach.

Corchorus olitorius leaves are very nutritious, it is rich in calcium, iron, protein, vitamin A, C and E, thiamin, riboflavin, niacin, folate, and dietary fibers. It is usually cooked as stew, forming a thick slimy syrup similar in consistency to okra usually taken with rice or other starchy staple.

It can almost grow anywhere in the India. Its is a hardy plant that is resistant to pests and requires little care. It can be found in the wild as it can also be grown in a farm.

It plants are tall, reaching 2-4 meters in height, having only a few side branches. Its leaves are alternate, simple, lanceolate, about 5-15 cm in length tapering to a pointed tip and has finely serrated margin. Corchorus olitorius flowers are yellow about 2-3 cm wide with five petals. The fruit is capsule like with plenty of small seeds inside.

Corchorus Olitorius Herbal Medicine - Health Benefits

Corchorus olitorius leaves being rich in vitamins and minerals generally promotes good health and well-being.

Corchorus olitorius leaves being rich in vitamin A can provide health benefits for good eyesight.

It contains vitamin E and other antioxidants. It is said to prevent wrinkles and promote youthful looking skin.

It is used to treat inflammation and pain such as arthritis, headache, stomach ache and others.

It is being rich in fiber helps to control blood pressure, cholesterol build-up, diabetes and prevents heart disease.

Corchorus olitorius leaves are rich in fiber and its slimy consistency when cooked is used to treat various digestive problems such as diarrhea, stomach ache, dysentery, constipation and ulcers.

It is also claimed that together with other herbs it can cure cancer.

Corchorus olitorius is an annual herb whose leaves and roots are used as herbal medicine and eaten as vegetable by local people in East Malaysia, India, Egypt, and Philippines (Zeghichi et. al., 2003). Traditionally, its leaves are used in the treatment of pain, fever, chronic cystitis and tumors (Abu-Hadid et. al., 1994). Its seeds have been reported to posses estrogenic activity (Sharf et. al., 1979) as well as contain high content of hydrogen cyanide and several cardiac glycosides (Negm et. al., 1980). Its extract has been reported to suppress transformation of the aryl hydrocarbon receptor induced by dioxins (Nishiumi et. al., 2005).

Its polyphenolic isolate has been reported to have antiobesity effect (Wang et. al., 2011).

Its aqueous extract has also been reported to have protective effect in arsenic-induced myocardial injury (Das et. al., 2010). However, due to dearth of information from literature on the effect of Co chorus olitorius on hematological and plasma biochemical parameters in albino rats, this study therefore aims at investigating the effect of aqueous extract of Corchorus olitorius on these aforementioned parameters in male albino rats.

Technology & Engineering, ISSN: 2319-7463 Vol. 2 Issue 11, November-2013, pp: (35-44), Available online at: www.erpublications.com Page | 37 ascites, pain, piles, and tumors. Elsewhere the leaves are used for cystitis, dysuria, fever, and gonorrhea. The cold infusion is said to restore the appetite and strength (Duke, 1983; Annon.,).

2. MATERIALS AND METHODS

2.1. Adsorbent

The natural plant material Corchorus Olitorius-L-Leaves, used in the present investigations was collected from Thiruvarur district the Leaves were washed with distilled water several times to remove the dirt and dust and was subsequently dried in a hot air oven at 110^{0}C. Afterward, carbonization of the Corchorus Olitorius-L-Leaves was carried out at 650^{0}C for 1 hour in a muffle furnace. A linsang nitrogen (purity 99.99%) flow of 150ml/min was maintained throughout the process of carbonization primary carbon was obtained on carbonization, which was afterward mixed with Zinchloride. Zinc chloride acts as a catalyst in the process. The primary carbon was activated at 1100°C for 6 hrs under optimized conditions to obtain activated nano carbon. The activated carbon was thereafter looked to room temperature in an insert atmosphere of nitrogen and washed with hot distilled water and 0.5 N Hydrochloric and until the pH of the material reached 7.0 the activated carbon was also dried in a hot air oven at 110^{0}C, ground and sieved to obtain the desired particular size (150µm) and stored in desiccators for further use.

2.3. Chemicals

All chemicals used of high purity commercially available Analar grade purchased from scientific equipment company trichy. Iron solution was prepared from $FeSO_4.7\ H_2O$ (2.489 g in 500 ml distilled water equivalent to one gram/liter). All experimental solutions were prepared by diluting the stock solution to the required concentration. The pH of each experimental solution was adjusted to the required initial pH value using dilute HCl (or) NaOH before mixing the adsorbent. The concentration of residual Iron(II) was determined with atomic absorption spectrophotometer (Perkin Elemer 2380).

2.4. Batch experiments

The effect of various parameters on the removal of Iron (II) onto ACONC was studied batch adsorption experiments were conducted at (30-60°C). For each experimental run, 50 ml of iron solution of known initial concentration and pH were taken in a 250 ml plugged conical flask. A 25 mg adsorbent dose is added to the solution and mixture was shaken at constant agitation speed (200 rpm) sample were withdrawn at appropriate time intervals (10-60 min) and the adsorbent was separated by filtration. The residual solutions were analyzed to determine the Iron(II) concentration.

The effect of dosage of adsorbent on the removal of Iron(II) was measured by contacting 50 ml of 50 mg/L of Iron(II) solution with 25 mg of ACONC till equilibrium was attained.

Adsorption equilibrium isotherm is studied using 25 mg of ACONC dosage per 50 ml of Iron(II) solution. The initial concentration were ranged from (25 to 125 mg/L) in all sets of

experiments. The plugged conical flask was shaken at a speed of 200 rpm for 60 minutes. Then the solution was separated from the mixture and analyzed for Iron(II) concentration. The adsorption capacity was calculated by using a mass equilibrium equation as follows:

$$q_e = (C_0 - C_e)\ V/M \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where C_0 and C_e being the initial iron concentration (mg/L) and equilibrium concentration, respectively V is the experimental volume of Iron(II) solution expressed in liters [L] and M is the adsorbent mass expressed in grams [g]. The Iron(II) ions percentage can be calculated as follows:

$$\%R = (C_0 - C_t) \times 100/C_0 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The effect of pH on the rate of adsorption was investigated using iron concentration of 25 mg/L constant ACONC dosage. The pH values were adjusted with dilute HCl and NaOH solution. The adsorbent – adsorbate mixture was shaken at room temperature using agitation speed (200 rpm) for 60 minutes. Then the concentration of Iron in solution was determined.

3. RESULT AND DISCUSSION

3.1 Characterization

The different chemical constituents of activated Corchorus Olitorius-L-Leaves are given in Table 1 along with some other characteristics. X-ray spectra of both adsorbents do not show any peak indicating the amorphous nature of activated Corchorus Olitorius-L-Leaves.

Table 1-Characteristics of the Adsorbent

Properties	ACONC
Particle size(mm)	0.0100
Density (g/cc)	0.2041
Moisture content (%)	0.3027
Loss in ignition (%)	0.0110
pH of aqueous solution	6.3000

3.2 Adsorption studies

Batch experiments were performed to investigate the adsorption process of Fe(II) by the ACONC. For each experimental run, 50 mL of Fe(II) solution of known concentration, initial pH, ionic strength and the amount of the ACONC were taken in a 250-mL stoppered conical flask. This mixture was agitated in a temperature-controlled shaking water bath at a constant speed of 200 rpm/min and certain temperatures. For adsorption equilibrium studies, Fe(II) solutions of different concentrations were contacted with a certain amount of ACONC under certain conditions for an hr insuring the equilibrium was achieved. The residual Fe(II) concentration was then measured and the amount of Fe(II) adsorbed onto ACONC was calculated from mass balance. Effects of contact time, adsorbent dosage, initial Fe(II) concentration, initial solution pH, ionic strength and temperature) on Fe(II) adsorption by ACONC were investigated. Adsorption kinetics was determined by analyzing adsorptive uptake of Fe(II) from aqueous solution at different time intervals [18]. The amount of Fe(II) adsorbed at time t, q_t (mg/g), was calculated using mass balance equation.

3.3. Effect of Contact Time on batch Adsorption of Iron(II) ions in Aqueous Solution

Figure 1 shows the effect of contact time on the adsorption of Iron(II) ions solution using activated nano carbon from Corchorus Olitorius-L-Leaves. The concentrations of iron(II) ions in solution were varied from 25mg/L to 125mg/L and batch adsorption was carried out with 25mg of activated Corchorus Olitorius-L-Leaves. The percentage of iron(II) ions adsorbed increased with time until equilibrium was reached for each concentrations. It is therefore evident from Fig 1 that at low concentration ranges the percent adsorption is high because of the availability of more reactive sites. At higher concentration of metal ion more and more surface sites are covered, the capacity of the adsorbent get exhausted due to non-availability of active surface sites. This leads to a fall in the percentage of metal ion adsorbed at higher concentration [19]. It was observed that the percentage adsorption of iron(II) ion rapidly reached equilibrium at 30 minutes of contact for 25mg/L concentration, it increased to 100% implied that iron(II) (Fe^{2+}) ion was completely removed from aqueous solution at this concentration.

3.4. Effect of Initial Concentration on the Adsorption of Iron(II) Ion in Aqueous Solution

The effect of initial concentration of iron(II) ions on adsorption of iron(II) ions using Corchorus Olitorius-L-Leaves activated nano carbon. Adsorption of irons (II) ions in solution increase significantly with reduction in the initial concentration of iron (II) ions in solution. The initial concentration of adsorbate varied from 25mg/L to 125mg/L. The rate of adsorption decreased from 92% - 70% as the concentration of iron (II) ions increased from 25mg/L to 125mg/L within 30 min of adsorption. This was expected and shows that there more reactive sites on the pore of Corchorus Olitorius-L-Leaves activated carbon.

3.5. Effect of Carbon Dosage on the Batch Adsorption of Iron(II) Ions in Aqueous Solution

Iron(II) ions in aqueous solution of known concentration was adsorbed using different carbon dosage of activated Corchorus Olitorius-L-Leaves ranging from 25mg – 125mg in 50 ml of stock solution of iron(II) ions . The effect of carbon dosage on the adsorption of iron(II) ions using activated carbon from waste Corchorus Olitorius - L- Leaves is presented in Fig 2. There was significant increase in the adsorption of iron(II) ions in solution as carbon dosage increased within adsorption time of 30min [14] reported similar findings during the removal of heavy metal adsorption by modified oak sawdust. This is due to the increased availability of active adsorption sites arising due to the increase in effective surface area resulting from the increases in dose of adsorbent or due to conglomeration of the adsorbent. Increasing the adsorbent dosage further, it was found that the optimum uptake of iron(II) ions requires about 250 mg of activated carbon from Corchorus Olitorius-L-Leaves to adsorb 100% iron(II) ions in aqueous stock iron(II) solution.

3.6. Effect of Particle Size on the Batch Adsorption of Iron(II) ions in Aqueous Solution

Effect of particle size of activated carbon produced from waste Corchorus Olitorius-L-Leaves on the batch adsorption of iron(II) ions in aqueous solution. The adsorption of iron(II) ions increased with reduction in particle size [19]. The shape reduction also revealed that particle size of activated Corchorus Olitorius-L-Leaves carbon has significant effect on the adsorption of iron(II) ions in aqueous solution for batch process. Smaller particle size (≤ 150 μm) adsorbed the highest amount of iron(II) ions within 30 mins of adsorption, hence for effective adsorption of iron(II) ions in aqueous solution using Corchorus Olitorius-L-Leaves.

3.7. Effect of pH on the Batch Adsorption of Iron(II) Ions in Aqueous Solution

Effect of pH on Adsorption of heavy metals: The pH of the wastewater is one of the imperative factors governing the adsorption of the metal ions. The effect of pH was studied from a range of 2 to 6 under the precise conditions (at optimum contact time of 60 min, 200 rpm shaking speed, with 25mg of the adsorbents used, and at a room temperature of 30°C). From Figure-3, with activated carbon from Corchorus Olitorius-L-Leaves used as adsorbent, it was observed that with increase in the pH (2-6.5) of the wastewater, the percentage removal of iron((II)) ions increased up to the pH 6.5 as shown above. At pH 6.5, maximum removal was obtained for metal ions, with 92.72% removal of Fe(II). The increase in percentage removal of the metal ions may be explained by the fact that at higher pH the adsorbent surface is deprotonated and negatively charge; hence attraction between the positively metal cations occurred [20].

3.8. Adsorption Models

The adsorption equilibrium data were further analyzed into two well known isotherm models via Freundlich and Langmuir models.

3.9.1 Freundlich model

The Freundlich model which is an indicative of surface heterogeneity of the adsorbent is described by the following equation.

$$\text{Log } q_e = \log k_f + 1/n + \log Ce \qquad (9)$$

Where K_f and $1/n$ are Freundlich constants associated with adsorption capacity and adsorption intensity respectively, The Freundlich

plots between $\log q_e$ and $\log C_e$ for the adsorption of Fe(II) were drawn. It was found that correlation efficient values were less than 0.99 at both the temperature studied indicating that Freundlich model was not applicable to the present study.

3.8.2. Langmuir model:

The adsorption isotherm was also fitted to Langmuir model. The Langmuir equation which is valid for monolayer adsorption on to a surface is given below.

$$1/q_e = 1/q_m + 1/q_{mb}C_e \quad (10)$$

Where q_e (mgg^{-1}) is the amount adsorbed at the equilibrium concentration C_e (mol L^{-1}), q_m (mgg^{-1}) is the Langmuir constant representing the maximum monolayer adsorption capacity and b (L mol^{-1}) is the Langmuir constant related to energy of adsorption. The plots $1/q_e$ as a function of $1/C_e$ for the adsorption of Fe (II) was found linear. Suggesting the applicability if Langmuir model in the present adsorption system. The correction coefficient (R^2 = 0.9926 and 0.9932 at 30 & 60°C respectively for Langmuir model) confirm good agreement between both theoretical models and our experimental results the values of the monolayer capacity (q_m) and equilibrium constant (b) have been evaluated from the intercept and slope of these plots and given in Table 3. It is adsorbent for the Fe(II) is comparable to the maximum adsorption obtained from the adsorption isotherms. These facts suggest that Fe(II) is adsorbed in the form of monolayer coverage on the surface of the prepared adsorbent [21]. Satisfactory fitting of the Langmuir model to the adsorption of Fe (II) on Activated Corchorus Olitorius - L- Leaves adsorbent.

3.10. Kinetics study

The Kinetic adsorption data were evaluated to understand the dynamics of the adsorption reaction in terms of the order of the rate constant batch experiments were conducted to explore the rate of Fe (II) adsorption by Corchorus Olitorius - L- Leaves as described in adsorption isotherms section at pH 6.5. Three Kinetic models were applied to the adsorption Kinetic data in order to investigate the behavior of adsorption process of Fe (II) onto the adsorbents. These models include the pseudo first order Kinetics (reversible or irreversible), the pseudo – Second – order and the intra particle diffusion models the linear form of reversible pseudo – first – order model can be formulated as:

$$\ln\left(q_e - q_t\right) = \ln q_e - k_1 x\, t \quad (7)$$

Where q_e (mol/g) and q_t (mole/g) are the amount of Fe (II) adsorbed at equilibrium and at time t, respectively and K_1 (min^{-1}) is the rate constant K_1 values were evaluated from the linear regression of $\ln(q_e-q_t)$ versus data. Linear from of irreversible pseudo first order model can be formulated as:

$$\ln\left(C_o / C_t\right) = K \times t \quad (8)$$

Where C_o (mg/l) is the initial concentration of Fe(II) and C_t (mg/l) is equilibrium concentration of Fe(II) at time't' respectively, and Kg min^{-1} is the rate constant Evaluation of data has been done using linear plot of ln (C_o/C_t) versus time. The Linear form of pseudo – Second – order equation can be formulated us.

$$t / q_t = 1 / K_2 q_e^2 + t / q_e \quad (9)$$

Where q_e and q_t are surface loading of Fe(II) at equilibrium and time 't' respectively and K_2 (g/mg/min) is the second – order rate constant, The Linear plot of t/q_t as a function of provided not only the rate constant K_2, but also an independent evaluation of q_e. The fitting of experiment al data to the pseudo – first – order and the pseudo – second- order equation seemed to be_quite good for where the calculated correlation coefficients (R^2) almost the same values.

3.11. The Elovich equation and intra-particle diffusion model

The Elovich model equation is generally expressed as

$$dq_t / d_t = \alpha \exp(-\beta q_t) \quad \ldots\ldots(10)$$

Where; α is the initial adsorption rate (mg g^{-1} min^{-1}) and β is the desorption constant (g/mg) during any one experiment. To simplify the Elovich equation. [24] assumed $\alpha\beta t >> t$ and by applying boundary conditions $q_t = 0$ at t= 0 and $q_t = q_t$ at t = t Eq.(10) becomes:

$$q_t = 1/\beta \ln(\alpha\beta) + 1/\beta \ln t \quad \ldots\ldots\ldots\ldots\ldots (11)$$

If Fe(II) ions adsorption fits with the Elovich model, a plot of q_t vs. ln(t) should yield a linear relationship with a slope of (1/β)and an intercept of (1/β)ln (αβ). The Elovich model parameters α, β, and correlation coefficient (γ) are summarized in table 6. The experimental data such as the initial adsorption rate (α) adsorption constant (β) and the correlation co-efficient (γ) calculated from this model indicates that the initial adsorption (α) increases with temperature similar to that of initial adsorption rate (h) in pseudo-second–order kinetics models[22]. This may be due to increase the pore or active site on the ACONC adsorbent.

For adsorption of Fe(II) on to Corchorus Olitorius-L-Leaves the obtained results represent more conformity to pseudo-second order model (R^2 =0.95), the initial adsorption rate $K_2q_e^2$ for Corchorus Olitorius-L-Leaves. Kinetic data for the adsorption of Fe(II) were also analyzed according to intra-particle diffusion model achieve can be formulated as [25].

$$Qt = k_{pt} 0.5 \qquad (12)$$

Where q_t is the amount of Fe(II) adsorbed (mg/g) at time t, and k_p (mg/g $min^{0.5}$) is the rate constant for intra – particle diffusion. Results are shown in table 6. Usually the plot of qt versus $t^{0.5}$ may be distinguished in two or more steps taking place during adsorption process including instantaneous adsorption stage by external mass transfer (first sharper portion), intra-particle diffusion which is the rate controlling stage (second portion as the gradual adsorption stage) and the final equilibration of age where the intra – particle diffusion starts to slow down due to extremely low solute concentration in solution (the third portion).

3.12. Adsorption Thermodynamics

The thermodynamic parameters for the adsorption of Fe(II) ions by Activated Corchorus Olitorius-L-Leaves were determined using the following equations:

$$K_D = q_e/C_e \qquad (13)$$

$$\Delta G^o = -RT \ln K_D \qquad (14)$$

$$\ln K_D = (\Delta S^o/R) - (\Delta H^o/RT) \qquad (15)$$

Where K_D is the distribution coefficient for the adsorption in g/L, ΔG^o is the Gibbs free energy in J/mol, R is the universal gas constant in J/mol K, T is the absolute temperature in K, ΔS^o is the entropy change in J/mol K and ΔH^o is the enthalpy change in kJ/mol [23]. The values of Gibbs free energy (ΔG^o) for various temperatures were calculated from the experimental data. The values of enthalpy change (ΔH^o) and entropy change (ΔS^o) were estimated from the slope and intercept of the plot of ln K_D Vs 1/T. The estimated thermodynamic parameters were tabulated and shown in table 5. But the negative values of Gibbs free energy change (ΔG^o) obtained for the adsorption of Fe (II) ions by Activated Corchorus Olitorius-L-Leaves at various temperatures had shown the spontaneous nature of the adsorption process.

The positive values of enthalpy change (ΔH°) obtained for the adsorption of Fe(II) ions by Activated Corchorus Olitorius-L-Leaves at various temperatures indicated that the adsorption reactions were endothermic. The positive values of entropy change (ΔS°) for the adsorption of Fe(II) ions by Activated Corchorus Olitorius-L-Leaves at various temperatures showed the increased randomness at solid liquid interphase during the sorption processes of Fe(II) ions on the adsorbent ACONC. This is a direct consequence of (i) opening up of structure of adsorbent beads (ii) enhancing the mobility and extent of penetration within the adsorbent beads and (iii) overcoming the activation energy barrier and enhancing the rate of intra-particle diffusion [25].

The adsorption of Fe(II) ions by Activated Corchorus Olitorius-L-Leaves slightly increased when temperature was raised up to 60°C. It might be due to the generation of new active sites on the adsorbent surface and also due to the increased rate of pore diffusion. But when the temperature was further raised, adsorption processes had decreased largely. It showed that the adsorption processes of Fe(II) ions by Activated Corchorus Olitorius-L-Leaves were exothermic reactions and physical in nature which involved the weak forces of attraction between the sorbate-sorbent molecules.

4. Desorption studies

Desorption studies help to elucidate the nature of adsorption and recycling of the spent

adsorbent and the metal ions. If the adsorbed metal ions can be desorbed using neutral pH water, then the attachment of the metal ion of the adsorbent is by weak bonds. The effect of various reagents used for desorption studies. The results indicate that hydrochloric acid is a better reagent for desorption, because we could get more than 92% removal of adsorbed metal ion. The reversibility of adsorbed metal ion in mineral acid or base is in agreement with the pH dependent results obtained. The desorption of metal ion by mineral acids and alkaline medium indicates that the metal ion was adsorbed onto the ACONC through physisorption as well as by chemisorptions mechanisms[20].

CONCLUSION

Kinetics of batch adsorption of iron(II) ions from aqueous solution using activated carbon from waste Corchorus Olitorius-L-Leaves has been investigated. The amount of iron(II) ions adsorbed was found to vary significantly with process parameters such as particle size, carbon dosage, initial concentration of adsorbate and contact time. The adsorption process follows Langmuir and Freundlich isotherms but a better sorption fit using Langmuir isotherm model was obtained indicating a monolayer formation over a surface of the material. The monolayer saturation capacity of 166.7 mg of iron(II) ions adsorbed per g of Corchorus Olitorius-L-Leaves activated carbon was obtained and found to be higher than monolayer saturation capacity of other adsorbent used for iron(II) ions adsorption. Adsorption kinetics was modelled using the pseudo first order, pseudo second order kinetic equations, and intra-particle diffusion models. Sorption kinetics showed good agreement of the experimental data the pseudo second order kinetic reaction is the rate controlling step with some intra particle diffusion taking place.

The high adsorption intensity of Corchorus Olitorius-L-Leaves activated carbon and its affinity for Iron(II) ions can help solve many adsorption challenges in the industry and in water purification processes.

ACKNOWLEDGEMENT

The authors sincerely thank the University Grants Commission, New Delhi for providing the fund from Major Research Project.

REFERENCE

[1] Chapman D., 1992. Water quality assessments: A guide to the use of biota, sediments and water in environmental monitoring. Kluwer Academic Publishers, Dortrecht, pp: 372.

[2] Letterman, R.D., 1999. American Water Works Association: Water Quality and Treatment. 5th ed., McGraw- Hill, New York.

[3] Vaaramaa, K. and J. Lehto, 2003. Removal of metals and anions from drinking water by ion exchange. Desalination., 155: 157-170.

[4] Ellis, D., C. Bouchard and G. Lantagne, 2000. Removal of iron and manganese from groundwater by oxidation and microfiltration. Desalination., 130: 255-264.

[5] bin Jusoh, A., W.H. Cheng, W.M. Low, A. Nora'aini, and M.J.M.M. Noor, 2005. Study on the removal of iron and manganese in groundwater by granular activated carbon. Desalination., 182: 347-353.

[6] Munter, R., H. Ojaste, and J. Sutt, 2005. Complexed iron removal from groundwater. Journal of Environ. Eng., 131: 1014-1020.

[7] Okoniewska, E., J. Lach, M. Kacprzak and E. Neczaj, 2007. The removal of manganese, iron and ammonium nitrogen on impregnated activated carbon. Desalination., 206: 251-258.

[8] Berbenni, P., A. Pollice, R. Canziani, L. Stabile, and F. Nobili, 2000. Removal of iron and manganese from hydrocarbon-contaminated groundwaters. Bioresour. Technol., 74: 109-114.

[9] Aziz, H.A., M.S. Yusoff, M.N. Adlan, N.H. Adnan, and S. Alias, 2004. Physico-chemical removal of iron from semiaerobic landfill leachate by limestone filter. Water Manag., 24: 353-358.

[10] Ahmedna, M., W.E. Marshall, A.A. Husseiny, R.M. Rao, and I. Goktepe, 2004. The use of nutshell carbons in drinking water filters for removal of trace metals. Water Res., 38: 1062-1068.

[11] El-Sherif, I. Y. and N.A. Fathy, 2011. Equilibrium and kinetic study of Cd(II) and Ni(II) ions removal by semi–carbonized/H_3PO_4 cotton stalks. EJEAF Che., 10: 2744-2758.

[12] Girgis, B.S., A.A. Attia, and N.A. Fathy, 2007. Modification in adsorption characteristics of activated carbon produced by H_3PO_4 under flowing gases. Colloids and Surfaces A: Physicochem. Eng. Aspects., 299: 79-87.

[13] Girgis, B.S., A.M. Soliman, and N.A. Fathy, 2011. Development of micro-mesoporous carbons from several seed hulls under varying conditions of activation. Microporous and Mesoporous Materials., 142: 518-525

[14] Kazemipour, M., M. Ansari, S. Tajrobehkar, M. Majdzadeh, and H.R. Kermani, 2008. Removal of lead, cadmium, zinc, and copper from industrial wastewater by carbon developed from walnut, hazelnut, almond, pistachio shell, and apricot stone. Journal of Hazardous Materials, 150: 322-327.

[15] Budinova, T., E. Ekinci, F. Yardim, A. Grimm, E. Björnbom, V. Minkova, and M. Goranova, Characterization and application of activated carbon produced by H_3PO_4 and water vapor activation. Fuel Processing Technology, 87: 899-905.

[16] Balintova, M. and A. Petrilakova, 2010. Study of pH influence on selective precipitation of heavy metals from acid mine drainage. In 14th Conference on Environmental and Mineral Processing, VSB-TU, Ostrava, Czech, Republic, pp: 160-166.

[17] Hamadi N K, Swaminathan S, Chen X D, 2004. Adsorption of paraquat dichloride from aqueous solution by activated carbon derived from used tires. Journal of Hazardous Materials, 112(1-2): 133–141.

[18] Shrivastava, R.K., Ayachi, A.K. and Mora, M. 2001. Removal of Cr (VI) by utilization of Bidi Stem. Pollut. Res. 20(4): 639 – 643).

[19] Namasivayam, C. and Holl, W.H. 2004. Chromium(III) removal in tannery wastewaters using Chinese reed (Miscanthus Sinensis), a fast growing plant. Holz. Roh. Werkst. 62: 74 – 80.

[20] Sharma, A. and Bhattacharya, D. 2005. Utilisation of biosorbent based on Azadirachta indica (Neem) Stem for removal of water soluble Metals. Indian J Environ. Chem. 12: 285-295.

[21] Singh, I.B. and Singh, D.R. 2001. Hexavalent chromium removal using iron bearing industrial sludges: Indian J. Chem. Technol. 8: 487-495.

[22] Jambulingam, M., Rehugadevi, N., Karthikeyan, S., Kiruthika, J., Patabhi. S. 2005. Adsorption of Cr(VI) from aqueous solution using a low cost activated carbon. Indian J Environ. Protect. 25(5): 458-63.

[23] Ngah W.S.W, Endud C.S, Mayanar R. (2002) Removal of copper(II) ions from aqueous solution onto chitosan and cross-linked chitosan beads React. Funct. Polym. 50, 181-190

[24] Chien S H, Clayton W R, "Application of Elovich Equation to the kinetics of Phosphate release and sorption on soil", Soil Sci. Sco, Am. J. 44 :265 – 268, 1980

[25] Weber W J, Morris J C, "Kinetics of adsorption on Carbon from solution". J, Sanitary Eng, Div. 90, 79, 1964.

TABLE: 2. EQUILIBRIUM PARAMETERS FOR THE ADSORPTION OF IRON (II) ION ONTO ACO NC

M_0	Ce (Mg / L)				Qe (Mg / L)				Removal %			
	30°C	40°C	50°C	60°C	30°C	40°C	50°C	60°C	30°C	40°C	50°C	60°C
25	4.4988	4.0353	4.5435	3.7325	91.565	94.926	95.902	96.523	92.515	93.356	94.365	94.256
50	10.435	10.465	8.4726	7.5651	167.12	169.04	173.05	174.86	88.125	89.145	91.145	92.158
75	22.746	22.364	19.641	18.363	238.55	245.27	260.76	265.24	82.235	85.368	86.214	88.952
100	44.844	41.648	22.644	36.678	314.32	312.78	350.71	316.68	76.458	78.456	90.365	81.324
125	71.882	66.936	43.648	60.907	332.25	350.12	408.78	368.19	70.658	72.125	83.690	75.562

TABLE: 3. LANGMUIR AND FREUNDLICH ISOTHERM PARAMETER FOR THE ADSORPTION OF IRON (II) ION ONTO ACONC

Temp. (°C)	Langmuir Parameters		Freundlich Parameters	
	Q_m	b	K_f	n
30°C	314.24	0.0356	1.4580	2.3645
40°C	319.58	0.0255	1.5601	2.2456
50°C	465.36	0.0425	1.6356	1.1255
60°C	337.20	0.0564	1.8562	2.1922

TABLE: 4. DIMENSIONLESS SEPERATION FACTOR (R_L) FOR THE ADSORPTION OF IRON (II) ION ONTO ACONC

(C_i)	Temperature °C			
	30°C	40°C	50°C	60°C
25	0.2825	0.2256	0.2456	0.1236
50	0.1235	0.1325	0.1456	0.1456
75	0.0912	0.0845	0.1022	0.0712
100	0.0801	0.0758	0.0823	0.0623
125	0.0622	0.0536	0.0623	0.0456

TABLE: 5. THERMODYNAMIC PARAMETER FOR THE ADSORPTION OF IRON (II) ION ONTO ACONC

(C_0)	ΔG°				ΔH°	ΔS°
	30°C	40°C	50°C	60°C		
25	-4786.1	-6446.5	-6802.7	-6992.1	6.1115	49.5121
50	-3319.1	-4798.5	-5878.6	-6068.4	14.962	53.6132
75	-2485.4	-3821.6	-4392.9	-4953.7	11.561	39.4712
100	-2150.9	-2459.8	-5293.1	-3194.0	15.582	49.3945
125	-1189.8	-1613.2	-3613.3	-2199.3	13.521	46.3884

TABLE: 6. THE KINETIC PARAMETERS FOR THE ADSORPTION OF IRON (II) ION ONTO ACONC

C_0	Temp °C	Pseudo second order				Elovich model			Intraparticle diffusion		
		q_e	k_2	γ	h	α	β	γ	K_{id}	γ	C
25	**30**	100.40	0.0018	0.994	19.52	818.20	0.0916	0.9959	1.7335	0.9981	0.1291
	40	100.17	0.0016	0.991	20.90	120.36	0.0951	0.9968	1.7504	0.9975	0.1225
	50	100.73	0.0014	0.992	20.87	190.19	0.1004	0.9982	1.7651	0.9969	0.1145
	60	100.07	0.0014	0.991	22.10	278.37	0.1052	0.9948	1.7738	0.9973	0.1092
50	**30**	190.41	0.0021	0.992	34.85	1260.1	0.0468	0.9961	1.7045	0.9989	0.1329
	40	192.37	0.0020	0.991	35.97	146.05	0.0471	0.9987	1.7145	0.9928	0.1302
	50	195.86	0.0018	0.993	38.53	196.87	0.0478	0.9967	1.7332	0.9941	0.1251
	60	197.17	0.0017	0.991	40.57	214.45	0.0477	0.9989	1.7400	0.9948	0.1242

C_0	Temp °C	Pseudo second order				Elovich model			Intraparticle diffusion		
		q_e	k_2	γ	h	α	β	γ	K_{id}	γ	C
75	30	266.65	0.0022	0.992	51.10	210.92	0.0341	0.9959	1.6825	0.9952	0.1296
	40	273.50	0.0022	0.994	52.60	227.99	0.0334	0.9984	1.6953	0.9983	0.1286
	50	280.24	0.0021	0.991	55.94	250.10	0.0328	0.9967	1.7095	0.9960	0.1276
	60	282.68	0.0020	0.992	47.44	460.59	0.0362	0.9983	1.7245	0.9940	0.1143
100	30	329.80	0.0024	0.994	54.09	115.09	0.0248	0.9943	1.6127	0.9946	0.1472
	40	338.49	0.0024	0.995	56.28	139.45	0.0248	0.9982	1.6317	0.9988	0.1429
	50	343.90	0.0011	0.997	59.50	167.36	0.0249	0.9972	1.6468	0.9954	0.1393
	60	351.13	0.0023	0.999	66.09	241.47	0.0254	0.9969	1.6716	0.9990	0.1324
125	30	385.32	0.0022	0.998	57.66	839.01	0.0199	0.9981	1.5570	0.9987	0.1600
	40	392.62	0.0025	0.997	61.02	103.48	0.0201	0.9948	1.5764	0.9967	0.1545
	50	405.44	0.0016	0.998	58.86	843.37	0.0188	0.9994	1.5756	0.9961	0.1609
	60	410.50	0.0025	0.992	65.92	128.62	0.0197	0.9972	1.6052	0.9952	0.1499

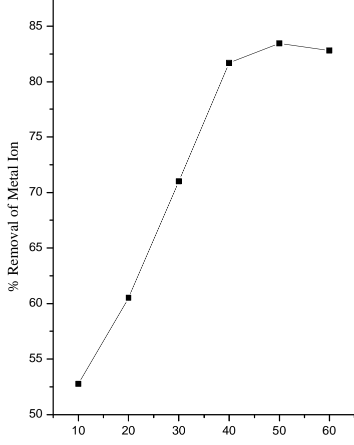


Fig:1-Effect of Contact Time on the Removal of Metal ion
[M]=50 mg/L; adsorbent dose=25mg/50ml; pH-6.5;Temp 30^{0}C

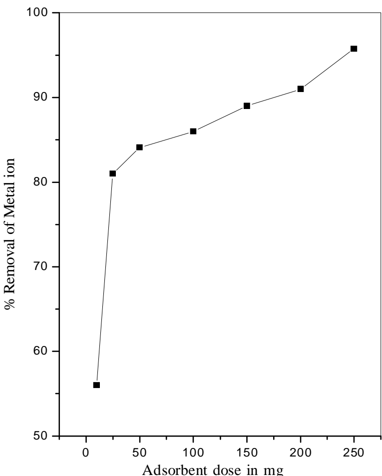


Fig.2-Effect of adsorbent dose on the removal of Metal Ion
[M]=50mg/L: Contact time=60 min: pH=6.5: Temp 30^{0}C

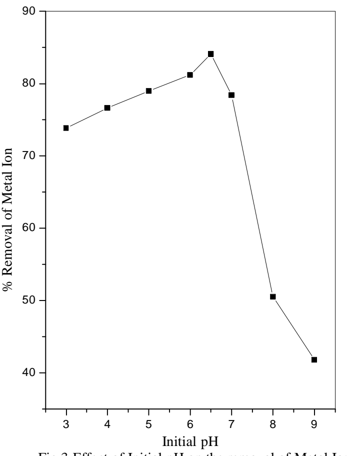


Fig.3-Effect of Initial pH on the removal of Metal Ion
[M]=50mg/L: Contact time=60 min: dose=25mg/50ml

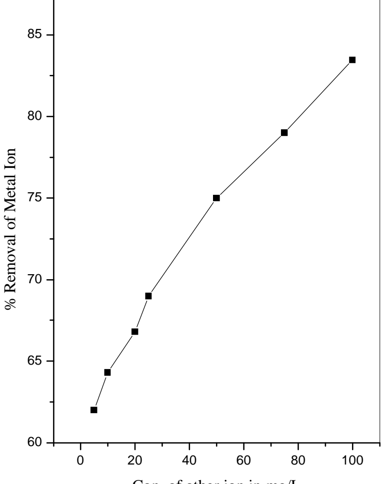


Fig.4-Effect ionic strength on the adsorption of Metal ion
[M]=50 mg/L;Contact time=60 min;Dose=25 mg/50 ml

RESEARCH UPDATE ON *CAESALPINIA BONDUC* (L.): A BRIEF REVIEW

Subbulakshmi. P*

Department of Pharmacology, Meenakshi Ammal Dental College and Hospital Maduravoyal, Chennai, India.

**Correspondence should be addressed to subbu.buvi@gmail.com*

Abstract

Therapeutic value of herbal plants plays a vital role in promoting positive health system in both humans and animals. Its restorative and rejuvenating power attracts the folks towards herbalism.

Caesalpinia bonduc (L.) (Caesalpiniaceae), commonly known as Nicker Nut. It's used in ayurvedic and siddha system of medicine for the treatment of various ailments. It is habitat throughout India and tropical countries of the world. All parts of the plant posses' curative power so thus considered as valuable traditional drug. Articles have been published reporting its antioxidant, hepatoprotective, antidiabetic, anxiolytic, larvicidal, muscle contractile, adaptogenic, nootrogenic properties. Research analysis encompasses the presence of numerous phytochemical constituents. This review endeavours the pharmacological characteristics,chemical constituents, traditional use and myth of the Caesalpinia bonduc available in literature. Recent updates and Directions for future research are also discussed.

Key words: *Caesalpinia bonduc (L.), Nicker Nut,remedy,phytochemicals,*

Introduction

Plants play a vital role in both humans and animals life from ancient days. The knowledge about the various functions of plants is communicated from generation to generation through frequent usage and by oral tradition (*Sagar and Vidyasagar, 2010*). Wider use of traditional plants helps to regulate proper health system and to determine the quality of life. Emerging use of medicinal plants and its products proves its caliber in maintaining the spectrum of living activities. World Health Organization (WHO) reported that 80 % of the world's population use medicinal plants as their main primary health care source in the treatment of diseases (*Ajose, 2007*). Abundant of conventional drugs have been used for treating numerous ailments in lungs, kidney, heart, brain, eye, gynecological, metabolic and various other disorders. Drug resistance, Cost and untoward effects of the synthetic drugs enable the folks to revisit the "Green medicine". Pharmacognostic and Ethanopharmacological updates reveal the use of herbalism has increased throughout the world.

Synonyms

Caesalpinia bonduc (L.); Caesalpinia Crista (L.); Caesalpinia paniculata, Guilandina paniculata . Besides species like ***Caesalpinia nuga*** and ***Caesalpinia jayoba*** are also sometimes wrongly designated as synonyms for ***C .crista***.

Common Names

Sanskrit : Kuberaksah, Kuberaksi
Tamil : Kalachi ver, Kalachikai,Kalichikai
English : Fever nut, Bonduc nut
Telugu : Mulluthige, Gachakaya
Malayalam : Kaka- moullou, Kazhanji
Kanada : Gajikekayi
Marathi : Gajaga
Konkani : Vakeri.

Description

***Caesalpinia bonducella* (L.),** commonly called **"**Nikker Nuts" belonging to the family: Caesalpiniaceae/Fabaceae. It is named in honor of Andreas Caesalpini, Italian botanist and chief physician to Pope Clement VIII. "Bonducella" the name of the species is derived from the Arabic word "Bonduce" means a "Little ball" which indicates the spherical shape of the seed *(Handa SS et al 1996).* All parts of the plant have medicinal properties so it is a very valuable traditional system of medicine (*Kirtikar et al. 1998*).

Distributed widely and specifically seen in India, Nepal, Indonesia, Polynesia, Srilanka, Andaman and Nicobar Islands. In India

specially viewed in Tropical regions *(Francis 2002; Asolkar et al.1992; White R. et al.2005).*

It is thorny shrub seen along the seashores as an extensive creepers. Leaves are large and bi-pinnate with dense flowers. Fruits inflated pod with wiry prickles' contains 1-2 seeds inside the pod. Seeds are large, round, shiny in gray color.

The pharmacological screening of the plant extracts reveals their anticancer, antioxidant, antimalarial, antihyperglycemic, anti-inflammatory, antirheumatic, antipyretic, anticonvulsant, antiameaobic, anti-estrogenic and abortifacient activities *(Adesina, 1982; Datté et al., 1998; Gupta et al., 2003; Chakrabarti et al. 2003; Gupta et al., 2004; Sonibare et al., 2009).*

Recently, (*Jäger and Saaby, 2011)* reported the anti-depressant, anti-anxiety, memory inducer and relaxing enhancer of *Caesalpinia bonduc*. The phytochemical analysis of the plant shows that it contains Saponins, Alkaloids, Flavonoids, Triterpenoids, Diterpenoids, Tannins and steroids (Kumar *et al*., 2005).

Pharmacological Features

Antioxidant Activity

Antioxidants used to prevent the oxidative stress by its ROS scavenging capability. Studies indicates that Ethanolic and Chloroform extract of *Caesalpinia bonducella* seeds posses natural antioxidant properties helps in inhibiting hydroxyl radical, nitric oxide, superoxide anions *(Shukla et al. 2009; Sachan et al. 2010)*

Anti-Inflammatory/ Analgesic / Antipyretic Activity

Inflammation is a pathophysiological response to cell damage which may leads to various diseases. Studies indicates that Methanolic Extract of *Caesalpinia bonducella* (MECB) seed acts as a potential source of an anti-inflammatory, antipyretic and analgesic agent.

Antidiabetic Activity

Most of the *Caesalpinia* Species shows antidiabetic and hypoglycemic properties. Different extracts of Bonduc seed kernels and powders used to treat diabetes by blocking the glucose absorption. It also involves in reducing Diabetes induced hyperlipidemia and Oxidative stress. *(Kannur et al, Pati et al 2010)*

Antimicrobial Activity

Studies indicates that *Caesalpinia bonducella* seed extract possess its activities against bacteria, Fungi. Alcoholic extracts of roots and stem showed antiviral activity against vaccinia virus *(Simin et al 2001;DharML et al 1968)*

Antipsoriatic and Antispasmodic Activity

Studies reported that Leaves of C*aesalpinia bonducella* have been used traditionally for treating Psoriasis *(Muruganantham et al 2011*). Its Antagonistic effect towards calcium ions helps as spasmolytic *(Khan et al 2011)*

Antiestrogenic Activity

Studies shows that alcohol seed extract of C*aesalpinia bonducella has* antiestrogenic property by inhibits the secretion of estrogen *(salunke et al. 2011).*

Anxiolytic Activity

Anxiety is a permanent state of worry and nervousness occurring in a variety of mental disorders. It is reported that seed extract of C*aesalpinia bonducella* exhibited dose dependent anxiolytic property in experimental animals.*(Ali et al. 2008).*

Antitumor Activity

Tests done on experimental rats using Methanolic extract of *Bonduc* leaves showed inhibitory effects against Ehrlich Ascites Carcinoma and decrease the tumor activity which helped to increase the life span of the organism *(Gupta et al 2004)*

Antiproliferative Activity

Cassane diterpenes is an isolated compound from C*aesalpinia bonduc* reported to inhibits the proliferative activity against **MCF-7**(Breast adenocarcinoma), **C33A** (Cervical carcinoma), **DU145** (Prostate carcinoma) and Vero (African green monkey kidney fibroblast) cells *(Yadav et al.2009)*

Hepatoprotective Activity

Screening on experimental rats reported that Methanol extract of C*aesalpinia bonduc* act as a potent hepatoprotective agent by decreasing the activity of serum enzymes, bilirubin, uric

acid and lipid peroxidation and significantly increased the levels of SOD, CAT, GSH, Vitamin C, Vitamin E in a dose dependent manner.*(Sampath et al)*

Immunomodulatory Activity

It is reported that Ethanolic extract of C*aesalpinia bonducella* increases the neutrophil count and antibody activity thus shows therapeutic significance in preventing auto immune disorder by its potential immunomodulatory potency. *(Shukla et al.2009)*

Antifilarial Activity

Filariasis is a parasitic disease caused by an infection by Microfilariae affects the skin, abdomen and lymphatic system. Study indicates that crude extract and seed kernel of C*aesalpinia bonducella* shows microfilaricidal, macrofilaricidal and female worm sterilizing efficacy *(Gaur et al2008)*.

Antihelminthic Activity

Helminthiasis, is a macroparasitic disease targeting gastro intestinal tract and induce physiological damage. Bark and leaves extract of C*aesalpinia bonducella* shows antihelminthic activity against Pheritima posthuma and Ascardia galli which is a traditional medicine in Pakistan *(Jabbar et al 2007, Wadkar et al 2010)*

Folklore utilities

Marble like *Bonduc* seeds contain bonducin, a white, bitter glycoside referred to as "poor man's quinine." used as a substitute for quinine in the treatment of intermittent malarial fever.

Seeds are considered to be styptic, tonic, purgative, antiblennorrhagic. Also it is useful in colic, hydrocele, skin disesase, leprosy and abortifacient. Caribbeans make a medicinal coffee or tea with roasted senna and *Bonduc* seeds as naturopathic remedies including colds and stomach disorders. Bonduc seed oil used in convulsions and paralysis. In guinea, pounded seeds considered vesicant. In west Indies the roasted seeds are used as anti diabetic *(WIO 1992; Komal et al.2010)*.Burnt seeds with alum and burnt arecanut used as a dentifrice in spongy gums and gum boils.

The root bark is emmenagogue, febrigue expectorant, anthelmintics. In Jamaica, it is used as rubifacient and as local application for sores *(Komal et al.2010)*. As an infusion they are used for curing cerebral haemorrhage and infantile convulsions. Finely powdered leaves are considered as a uterine tonic after child birth. In Sri Lanka, in the indigenous system of medicine, the plant is used for treatment of skeletal fractures. In Hawaii islands, pod pulp used as blood purifier and as a laxative.

Stem used in eye diseases and as a fish poison. Seeds, leaves and roots are used for treating tachycardia, bradycardia, tuberculosis, tympanitis, pain in the abdomen, fever, cold and liver fluke in ruminants.

Grow wild on beaches of many Caribbean Islands and the seeds are commonly collected and strung into bracelets and necklaces as ornamental beads.

Pulverized bonduc seeds mixed with egg white and tulsi leaf extract may reduce the PCOD is a kind of oral tradition medicine in Tamilnadu.

Recent updates on *Caesalpinia bonducella*

New compound caesanol, diterpene 6β, 7β-dibenzoyloxyvouacapen-5α-ol were elucidated by spectroscopic analysis from Ethanol extract of aerial part of *Caesalpinia bonduc. (Sarah et al 2014).*

Six new cassane diterpenoids, named caesalls were isolated from the seed kernels of *Caesalpinia bonducella*. Structures were illuminated on the basis of spectroscopic analysis, mainly by NMR an MS. *(Lin Wu et al, 2014)*.

A new cassane diterpene neocaesalpin and triterpene, β-amyrin isolated from the seeds of *Caesalpinia bonduc (*Fabaceae) determined by NMR spectral methods. (Zhaohua Wu et al 2007).

Pharmacological trials have revealed diuretic and anti-pyretic activity of the nuts, and have also proved efficacious in diarrhea. In chennai an ointment is made from the powdered seeds with castor oil and applied externally in hydrocele and orchitis *(Handa et al.1996)*.

Conclusion

Research activities show the importance of *Bonduc* seeds in various pharmacognostic

studies. Bioactive compounds have been isolated from all parts of the plant. These parameters help in evaluating and standardization of the crude drug. Future Trials has to be recommended to develop and confirm the traditional oral medication in to proven studies which assist to include in various pharmacopeias.

Reference

Arif T, Mandal TK, Kumar N, Bhosale JD, Hole A, Sharma GL, Padhi MM, Lavekar GS, Dabur R. *In vitro* and *in vivo* antimicrobial activities of seeds of *Caesalpinia bonduc* (Lin.) Roxb. J Ethnopharmacol. 2009; 123:177-80.

Ata A, Udenigwe CC, Gale EM, Samarasekera R. Minor chemical constituents of *Caesalpinia bonduc*. Nat Prod Commun. 2009; 4:311-4.

Bhattacharyya A, Rai S, Babu CR. A trypsin and chymotrypsin inhibitor from *Caesalpinia bonduc* seeds: isolation, partial characterization and insecticidal properties. Plant Physiol Biochem. 2007; 45:169-77.

Datté JY, Traoré A, Offoumou AM, Ziegler A. Effects of leaf extract of *Caesalpinia bonduc* (Caesalpiniaceae) on the contractile activity of uterine smooth muscle of pregnant rats. J Ethnopharmacol. 1998; 60:149-55.

Datté JY, Yapo PA, Kouamé-Koffi GG, Kati-Coulibaly S, Amoikon KE, Offoumou AM. Leaf extract of *Caesalpinia bonduc* Roxb. (Caesalpiniaceae) induces an increase of contractile force in rat skeletal muscle in situ. Phytomedicine. 2004; 11:235-41.

Erharuyi O, Engel-Lutz N, Ahomafor J, Imieje V, Falodun A, Nebe B, Langer P. Anticancer activity of five forest crops used in African folklore: antiproliferative and pro-apoptotic effects. Nat Prod Res. 2014. [Ahead of print]

Gacche RN, Dhole NA. Aldose reductase inhibitory, anti-cataract and antioxidant potential of selected medicinal plants from the Marathwada region, India. Nat Prod Res. 2011; 25:760-3. Muruganantham N, Basavaraj KH, Dhanabal SP, Praveen TK, Shamasundar NM, Rao KS. Screening of *Caesalpinia bonduc* leaves for antipsoriatic activity. J Ethnopharmacol. 2011; 133:897-901.

Hullatti K, Pathade N, Mandavkar Y, Godavarthi A, Biradi M. Bioactivity-guided isolation of cytotoxic constituents from three medicinal plants. Pharm Biol. 2013; 51:601-6.

Jadhav AN, Kaur N, Bhutani KK. A new furanoditerpenoid marker for the distinction between the seeds of two species of Caesalpinia. Phytochem Anal. 2003; 14:315-8.

Jana K, Chatterjee K, Ali KM, De D, Bera TK, Ghosh D. Antihyperglycemic and antioxidative effects of the hydro-methanolic extract of the seeds of *Caesalpinia bonduc* on streptozotocin-induced diabetes in male albino rats. Pharmacognosy Res. 2012; 4:57-62.

Jana K, Chatterjee K, Ali KM, Ghosh A, Bera TK, Ghosh D. Antioxidant potential of hydro-methanolic extract of seed of *Caesalpinia bonduc*: An *in vitro* study. J Adv Pharm Technol Res. 2011; 2:260-5.

Kannur DM, Hukkeri VI, Akki KS. Adaptogenic activity of *Caesalpinia bonduc* seed extracts in rats. J Ethnopharmacol. 2006; 108:327-31.

Kannur DM, Paranjpe MP, Sonavane LV, Dongre PP, Khandelwal KR. Evaluation of *Caesalpinia bonduc* seed coat extract for anti-inflammatory and analgesic activity. J Adv Pharm Technol Res. 2012; 3:171-5.

Kinoshita T. Chemical studies on the Philippine crude drug calumbibit (seeds of *Caesalpinia bonduc*): the isolation of new cassane diterpenes fused with alpha,beta-butenolide. Chem Pharm Bull (Tokyo). 2000; 48:1375-7.

Kumar SR, Krishna V, Venkatesh, Pradeepa K, Kumar KG, Gnanesh AU. Direct and indirect method of plant regeneration from root explants of *Caesalpinia bonduc* (L.) Roxb.--a threatened medicinal plant of Western Ghats. Indian J Exp Biol. 2012; 50:910-7.

Lyder DL, Peter SR, Tinto WF, Bissada SM, McLean S, Reynolds WF. Minor cassane diterpenoids of *Caesalpinia bonduc* . J Nat Prod. 1998; 61:1462-5.

Mandal EK, Mandal S, Maity S, Behera B, Maiti TK, Islam SS. Structural studies of an immunostimulating gluco-arabinan from seeds of *Caesalpinia bonduc*. Carbohydr Polym. 2013; 92:704-11.

Neamsuvan O, Tuwaemaengae T, Bensulong F, Asae A, Mosamae K. A survey of folk remedies for gastrointestinal tract diseases from Thailand's three southern border provinces. J Ethnopharmacol. 2012; 144:11-21.

Ogunlana OO, Ogunlana OE, Adeneye AA, Udo-Chijioke O, Dare-Olipede T, Olagunju JA, Akindahunsi AA. Evaluation of the toxicological profile of the leaves and young twigs of *Caesalpinia bonduc* (Linn) roxb. Afr J Tradit Complement Altern Med. 2013; 10:504-12.

Prasad GP, Naidu ML. Effect of Kuberaksha Patra Churna, Vriddhadaru Mula Churna and Kandughna Taila in Shlipada (Manifested filariasis). Ayu. 2012; 33:102-9.

Prasad GP, Trimurtulu G, Reddy KN, Naidu ML. Analytical study of Kuberaksha/Kantaki Karanja Patra Churna [*Caesalpinia Bonduc* (L.) Roxb. Leaf powder]. Ayu. 2010; 31:251-4.

Pudhom K, Sommit D, Suwankitti N, Petsom A. Cassane furanoditerpenoids from the seed kernels of *Caesalpinia bonduc* from Thailand. J Nat Prod. 2007; 70:1542-4.

Quiroz D, Towns A, Legba SI, Swier J, Brière S, Sosef M, van Andel T. Quantifying the domestic market in herbal medicine in Benin, West Africa. J Ethnopharmacol. 2014; 151:1100-8.

Saravanan KS, Periyanayagam K, Ismail M. Mosquito larvicidal properties of various extract of leaves and fixed oil from the seeds of *Caesalpinia bonduc* (L) Roxb. J Commun Dis. 2007; 39:153-7.

Shameel S, Usmanghani K, Ali MS, Ahmad VU. *Caesalpinia bonduc* (L.) Roxb. Seed oil: lipid composition assessment. Pak J Pharm Sci. 1997; 10:29-38.

Shukla S, Mehta A, Mehta P, Vyas SP, Shivaprasad HN. *In vivo* immunomodulatory activities of the aqueous extract of bonduc nut *Caesalpinia bonducella* seeds. Pharm Biol. 2010; 48:227-30.

Simin K, Khaliq-Uz-Zaman SM, Ahmad VU. Antimicrobial activity of seed extracts and bondenolide from *Caesalpinia bonduc* (L.) Roxb. Phytother Res. 2001; 15:437-40.

Udenigwe CC, Ata A, Samarasekera R. Glutathione S-transferase inhibiting chemical constituents of *Caesalpinia bonduc*. Chem Pharm Bull (Tokyo). 2007; 55:442-5.

Wu L, Luo J, Zhang Y, Wang X, Yang L, Kong L. Cassane-type diterpenoids from the seed kernels of *Caesalpinia bonduc*. Fitoterapia. 2014; 93:201-8.

Wu L, Wang X, Shan S, Luo J, Kong L. New cassane-type diterpenoids from *Caesalpinia bonduc*. Chem Pharm Bull (Tokyo). 2014; 62:729-33.

Yadav PP, Maurya R, Sarkar J, Arora A, Kanojiya S, Sinha S, Srivastava MN, Raghubir R. Cassane diterpenes from *Caesalpinia bonduc*. Phytochemistry. 2009; 70:256-61.

"GC-MS ANALYSIS OF METHANOL EXTRACTS OF FLOWERS OF *ALLAMANDA NERIIFOLIA HOOK*"

R. Sumathi
Dept of Biochemistry, RA College for Women, Tiruvarur, Tamilnadu
Author for correspondence; E-mail: sumathiraguraman@ymail.com,

ABSTRACT

In this Study, the phytochemical constituents of Allamanda neriifolia hook flowers have been evaluated using GC-MS analysis. The chemical compositions of the Methanol extracts of flowers of Allamanda neriifolia hook were investigated using Perkin-Elmer Gas Chromotrophy-Massspectroscopy. The GC-MS analyses determined the presence of 68 different phytochemical compounds. This study helps to predict the formula and structure of biochemical which have been used as drugs and further investigation may lead to the development of drug formulation.

Key Words: *GC-MS analysis, phytochemical compounds, Methanol extracts, Allamanda neriifolia Hook.*

INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative health care since immemorial. Bioactive compounds derived from plant extracts have been reported scientifically for biological activities. Plant produce phytochemicals to protect themselves; but recent studies indicate that many phytochemicals can also protect humans against infectious dieases [1]. These are innumerable potentially useful Medicinal plants and herbs waiting to be evaluated and exploited for their effective biological activities. The family Apocynaceae consists of several important medicinal plants with wide range of biological activities and interesting phytochemical constituents. *Allamanda neriifolia Hook* commonly known as the Yellow Bell, Golden Trumpet or The Buttercup flower is agenus of tropical shrubs and vines belonging to the family Apocynaceae. It has been used as a purgative or emetic, febrifuge as well as for the treatment of coughs, headaches, jaundice and enlarged spleen resulting from malaria. The milky sap is also known to posses antibacterial and possibly anticancer properties [2].

Thus as the experimental plant species posses immense medicinal properties , therefore the aim of the study is to identify the biochemical compounds of *Allamanda neriifolia Hook* by using the methanolic flower extract through Gas Chromotography-Mass spectrum analysis.

MATERIALS AND METHODS

Collection and preparation of plant material

The fresh flowers of plant A.*neriifolia Hook* was collected from the natural habitates of Tiruchirappalli district, Tamil Nadu, India. The samples were washed individually under running tap water to remove any traces of soil particles and other dirt. They were dried at 60°C for 2 days in an oven, and then macerated to powder form with a mixer grinder.

Preparation of sample for GC/MS study

About 20 grams of the powdered flowers of samples of A.*neriifolia Hook* were soaked in 100 ml methanol individually. It was left for 24 hours so that alkaloids, flavonoids and other constituents if present will get dissolved. The methanol extract was filtered using Whatman No.1 filter paper and the residue was removed. It was again filtered through sodium sulphate in order to remove the traces of moisture.

Gas chromatography – Mass Spectrum analysis

The Concentrated Methanolic extracts of flowers of A.*neriifolia Hook* were analyzed using the clarus 500 GC-MS (Perkin Elmer) equipment at the Periyar Pharmaceutical College, Thiruchirappalli, Tamil nadu, India. About 1µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. As the compounds were separated, they eluted from the column

and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the Retention time (RT).While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass. The M/Z (Mass / Charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule.

Before analyzing the extract using Gas Chromatography and Mass Spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV.The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane).

Identification of components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08. LIB [3], WILEY8. LIB [4] library sources were used for matching the identified components from the plant material.

RESULTS

The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the methanolic extracts of *Allamanda neriifolia Hook* flowers. The compounds with their retention time (RT), Molecular formula, molecular weight (MW) and concentration (peak area) are presented in Table 1. The GC-MS chromatogram of the various peak of the compounds detected was carried out in methanolic flower extracts of *Allamanda neriifolia Hook are*, shown in Fig1. In the GC-MS analysis, 68 bioactive phytochemical compounds were identified. The identification of phytochemical compounds is based on the peak area, retention time, molecular weight and molecular formula. The highest peak area of 3.83 was obtained by both 2,3-Dihydro 3,5- dihydroxy-6-methyl-4H-pyran-4-one and 3,5-dihyroxy-2—methyl-5,6-dihydro pyran with retention time 16.780 and the lowest peak area of 0.42 was obtained by both phenol and 6-hydroxy hexan with retention time 10.952. Hexadecanoic acid, Pentadecanoic acid (n-alkanoic acids), Beta-Carotene, tocopherol (vitamin-E) and squalene, 1-Eicosanol (tri-terpene), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Terpene alcohol) also observed.

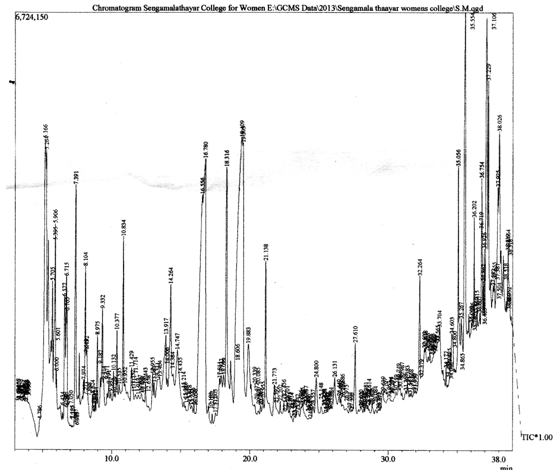


FIG – 1:GC – MS Spectra of methanolic extract of flowers of *allamanda neriifolia Hook*

TABLE – 1: Phyto components identified in the methanolic extracts of flowers of A*llamanda nerifoila Hook by* GC – MS analysis

S. No.	R.T	Name of compound	Peak Area %	M.F	M.Wt
1	5.166	Methylhydrazine	3.71	C H6 N2	46
2	5.166	Bilorin	3.71	C H2 N2	46
3	5.166	Fluoroethylene	3.71	C2 H3 F	46
4	5.166	2,2,4,4-D4-cyclobutanol	3.71	C4 H4 D4 O	72
5	5.166	1-dioxid	3.71	C2 H2 O3 S2	138
6	5.166	Methylphosphine	3.71	C H5 P	45
7	5.264	Ethylic acid	0.92	C2H4O2	60
8	5.264	Peracetic Acid	0.92	C2H4O3	76
9	5.395	2-Propanone, 1-hydroxy-	1.55	C3H6O2	74
10	5.395	1,2-Ethanediol,	1.55	C4H8O3	104
11	5.395	Methyl ethyl Ketone	1.55	C4H8O	72
12	5.601	1,2,3- Propanetriol	0.86	C3H8O3	92
13	5.705	2-Propenoic acid,	0.89	C4H6O2	86
14	5.705	Ethylene glycol diacrylate	0.89	C8H10O4	170
15	5.906	Propanoic acid, 2-oxo-, methyl ester Methyl pyruvate	1.68	C4H6O3	102
16	5.906	l - Chloroethyl acetate	1.68	C4 H5 CL O2	120
17	5.906	Hydrazoic acid	1.68	H N3	43
18	5.906	3-Chloro-3-fluoro-2-pentanone	1.68	C5 H8 CL FO	138
19	5.906	3-METHOXY-1-METHYL-CYCLOHEXENE	1.68	C6 H9 N O4	159
20	7.391	2-Furanmethanol	1.75	C5 H6 O2	98
21	7.391	2-DEUTEROXYMETHYL FURAN	1.75	C5 H5N D O2	98
22	7.888	Isopropenylacetic Acid	0.46	C5 H8 O2	100
23	8.104	2-Cyclopentene, -1, 4-dione	0.88	C5 H4 O2	96
24	8.104	4(3H)-Pyrimidinone	0.88	C4 H4 N2 O	96
25	8.975	2(5H)-FURANONE	0.78	C4 H4 O2	84
26	8.975	1-Methyltetrazole	0.78	C2 H4 N4	84
27	9.332	1- Piperidinepropionitrile	0.87	C8 H14 N2	138
28	9.332	2-Hydroxy-2-cyclopenten-1	0.87	C5 H6 O2	98
29	9.332	CYCLOOCTANONE 2-Brome	0.87	C8 H13 BR O	204
30	10.377	5-Methyl-2-furfural	0.81	C6 H6 O2	110
31	10.952	Phenol	0.42	C6 H6 O	94
32	10.952	6-HYDROXY-HEXAN-2	0.42	C6 H7 15N	93
33	10.952	4-hydroxy-Benzenesulfonic acid	0.42	C6 H6 O4 S	174
34	11.429	2H-Pyran-2, 6(3H)-dione	0.61	C5 H4 O3	112
35	12.955	PROPAN	0.55	C6 H9 N O2	127
36	13.917	HYDROXY DIMETHYL FURANONE	1.63	C6 H8 O3	128
37	13.917	2,5- Dimethyl -4-hydroxy-3(2H)-furanone	1.63	C6 H8 O3	128
38	16.556	N-Methyl-N-nitroso 2- Propanamine	4.51	C4 H10 N2 O	102
39	16.780	2,3-Dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one	3.83	C6 H8 O4	144
40	16.780	3,5-dihydroxy-2-Methyl-5,6-Dihydropyran Flavonoid	3.83	C6 H8 04	144
41	18.316	2-3-DIHYDRO-BENZOFURAN	2.27	C8 H8 O	120

S. No.	R.T	Name of compound	Peak Area %	M.F	M.Wt
42	18.316	4-vinylphenol	2.27	C8 H8 O	120
43	18.316	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-	2.27	C9 H8 O3	164
44	19.409	2,5-Di (hydroxymethyl)-furan	3.63	C6 H8 O3	128
45	19.503	2-Furancarboxaldehyde, 5-	1.64	C6 H6 O3	126
46	21.138	2-methoxy-4-etheny phenol	1.05	C9 H10 O2	150
47	21.138	4-vinyl-2-methoxy-phenol	1.05	C9 H10 O2	150
48	21.318	1-Hydroxy-2-acetyl-4-methylbenzene	1.05	C9 H10 O2	150
49	21.318	2-METHOXY-6-VINYLPHENOL	1.05	C9 H10 O2	150
50	27.610	Dodecanoic acid	0.51	C12 H24 O2	200
51	32.264	Tetradecanoic acid	0.82	C14 H28 O2	228
52	32.264	Eicosanoic acid	0.82	C20 H40 O2	312
53	32.264	Pentadecanoic acid	0.82	C15 H30 O2	242
54	32.602	Hexadecanoic acid	0.51	C16 H32 O2	256
55	33.704	M0ME INOSITOL	0.93	C7 H14 O6	194
56	35.056	Methyl palmitate	1.16	C17 H34 O2	270
57	35.267	GLYCEROL	0.50	C39 H72 O5	621
58	36.202	GERANYL LINALOOL ISOMER	0.81	C20 H34 O	290
59	36.202	1,6,10,14,18,22-TETRACOSAHEXAEN-3-OL	0.81	C30 H50 O	426
60	36.202	3,7,11,15-TETRAMETHYL-HEXADECA-1,6,10,14-TETRAEN-3-OL	0.81	C20 H34 O	290
61	36.202	FARNESYL ACETATE	0.81	C17 H28 O2	264
62	36.202	3,7,11,-TRIMETHYL-2,6,10-DODECATRIEN-1-YLJESTER	0.81	C17 H28 O2	264
63	36.202	SOLANESOL	0.81	C45 H74 O	631
64	36.202	d-Nerolidol	0.81	C15 H26 O	222
65	36.202	(E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	0.81	C20 H32	272
66	36.202	6,10,14,18,22-TETRACOSAPENTAEN-2-OL,	0.81	C30 H51 BR O	506
67		(ALL-E			
68	37.925	Allocryptopine	1.56	C21 H23 N O5	369

DISCUSSION

Medicinal plants are used in traditional treatments to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal medicine. Thus, for the above mentioned reason and bearing in mind its medicinal importance, the plant species *Allamanda neriifolia Hook* were selected to analyze by GC-MS technique and to explore the major and minor phytoconstituents present in the respective plant species.

The preliminary phytochemical screening shows the presence of saturated and unsaturated fatty acids, hydrocarbons, glycosides, polysaccharides, phenolic compounds, alkaloids, flavonoids, phytosterols, monoterpenes, triterpenes, sesquiterpenes, essential oil and vitamins. The prediction of the biological activities by applyingthe Duke's databases was confirmed with previous observations and supplemented the traditional usage of the *A. Neriifolia hook* [5-12]. By interpreting these compounds, it is found that *A. Neriifolia hook* possess various therapeutic application. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate

the relative concentrations of the components present in *A. Neriifolia hook*. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The presence of phytochemicals have been shown to possess antifungal, anti-candidal, allergenic, anesthetic, antibacterial, antipyretic, antiseptic, insectifuge, analgesic, anthelmintic, antioxidant, antispasmodic, antiinflammatory, diuretic, laxative, nephrotoxic, antitumor, anticancer, immunostimulant, chemopreventive and nematicide [13]. Hence, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of *A. Neriifolia hook*. The present work and detection of compounds is in consonance with the work reported by other scientists in different plant species viz., *Minuartia meyeri [14] Mentha spicata* and *Camellia sinensis [15], Kalanchoe pinnata [16]*.

CONCLUSION

It could be concluded that *A. Neriifolia hook* contains various bioactive compounds. So it is recommended as a plant of phytochemical importance. However further studies will need to be taken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and agricultural products.

REFERENCES

1. Hasan P, Yasa N, Ghanbari SV, Mohammadirad A, Dehghan G, Abdollahi M. In vitro antioxidant potential of Teucrium rolium, as compared to a-tocopherol. Acta Pharm 2007; 57: 123-129.
2. Tiwari TN, Pandey VB, Dubey NK. Plumieride from Allamanda cathartica as an antidermatophytic agent. Phytother Res 2002; 16(4): 393-394.
3. Lafferly MFW: Registry of mass spectral data, Edition. 5. Wiley New York; 1989.
4. Stein SE: National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02, USA; 1990.
5. Hema R, Kumaravel S, Alagusundaram K. GC/MS determination of bioactive components of Murraya koenigii. J Am Sci 2011; 7(1):80-83.
6. Praveen Kumar P, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. Afr J Biochem Res 2010; 4(7): 191-195.
7. Maruthupandian A, Mohan VR. GC-MS analysis of some bioactive constituents of Pterocarpus marsupium Roxb. Int J Chem Tech Res 2011; 3(3): 1652-1657.
8. Purabi Roy, Sarika Amdekar, Kumar A, Singh V. Preliminary study of the antioxidant properties of flowers and roots of Pyrostegia venusta (Ker Gawl) Miers. BMC Compl Alt Med 2011; 11: 11-69.
9. Ezhilan B, Neelamegam R. GC-MS Determination of bioactive compounds of Polygonum glabrum (Wild). J Phytol 2011; 3(9): 23- 25.
10. Hemalatha RG, Padmini E. Studies with the latex of the milk wed- Calotropis procera (Ait) R.Br. for spermicidal effect in human. J Pharm Res 2011; 4(2): 304-307.
11. Senthilkumar G, Madhanraj P, Panneerselvam A. Studies on the compounds and its antifungal potentiality of fungi isolated from paddy field soils of Jenbagapuram Village, Thanjavur District, and South India. Asian J Pharm Res 2011; 1(1): 19-21.
12. Hema R, Kumaravel S, Alagusundaram K. GC-MS Study on the bioactive components and anti-cancer activities of Solanum surattense. Cancer Biol 2011; 1(1): 13-17.
13. [Onlince] Available from: http://www.ars-grin.gov/duke/
14. Majaz Q, Nazim S, Shaikh S, Gomase P and Choudhari A Phytochemical Analysis Of Chloroform Extract Of Roots Of *Kalanchoe pinnata* By HPLC and GCMS**.** Int J Pharma Sci Res 2011; 2(7): 1693-1699.
15. Yayli N, Gulec C, Osman UC, Yasar A, Ulker S, Coskuncelebi K and Terzioglu S: Composition and Antimicrobial Activities of Volatile components of *Minuartia meyeri***.** Turk J Chem 2006. 30: 71- 76.
16. Admini E, Valarmathi A, Usha Rani M Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camellia sinensis*. Asian J Exp Biol Sci 2010; 1(4): 772- 781.

ECO-FRIENDLY DYEING OF COTTON FABRIC USING THE NATURAL MORDANTS

S. Thiyagarajan[1], K. Balakrishnan[2] & S. Tamilarasi[3]
Department of chemistry, A. V. V. M. Sri Pushpamcollege, Poondi, Thanjavur.

INTRODUCTION

Now a days, skin allergies due to clothes are becoming common. One primary reason for this is the use of non-eco-friendly dyes on textiles. Therefore, the European Community has banned few azo dyes that give bright colours on textiles. This has encouraged the revival of natural dyes globally. India, once famous for production of dyed and printed textiles from natural dyes is trying to revive its fast glory. Being biodegradable and rightly compatible with environment, the natural dyes are free from the effects associated with synthetic dye synthetic dyes such as harmfulness to human body, Pollution and waste water problems. The synthetic chemical dye causes many adverse effects on the skin and also environmental unfriendly since, natural dying methods used by the people needs revival, therefore this study was undertaken and was focused on dying properties of flowers of *Hibicusvittifolius.L, Fioriavitifolia(L.) Mattei.*Local Name: Siruthutthi

1.0. Materials and Methods

1.1. Materials

1.1.1. Source

Hibiscus vitifolius L. Undershrub, up to 1 m high; stem stellate-pubescent, greenish-purple. Leaves alternate, lower 3-5-lobed, upper merely angled, cordate to broadly ovate, 6x5-8, cordate at base, serrate at margin, acute-acuminate at apex, stellate-pubescent, glaucous beneath; palmately 3 - 5-nerved; petiole 2.5 - 11 cm long. Stipule small, deciduous.Flowers ca. 5 cm across, golden yellow, with dark purple center, solitary, axillary.Epicalyx linear-subulate, 1 cm long.Calyx cupshped, 1.5 mm across, lobe 5, ovate, 1 cm long, acute at apex. Corolla campanulate petals 5, broadly obovate, 4 cm long, purple at base. Staminal sheath, 1.8 cm long.Stamens many; filament slender, 2.5 mm long; anther 01 mm across. Ovary globose-ovoid, 05 x 04 mm long, hairy; 5-loculed; ovules 3 per cell; style 5 branching, thick, hairy; 3.5 mm long; stigma terminal, capitate, 0.5 mm long. Shizocarpusglobose, 1.5 cm across, stellate-pubescent, beaked at apex; seeds 3 per cell, trigonous-subreniform, 03 mm long, blackish.

The flowers of *Hibicusvittifolius.L, Fioriavitifolia (L.) Mattei.*was collected from alivalam village, Thanjavur district (fig.1).

Hibicusvittifolius.L, Fioriavitifolia(L.) Mattei. Fig: 1

Plant Name	:	*Hibiscus vitifolius L.*
Synonym	:	*Fioriavitifolia (L.) Mattei*
Family	:	Malvaceae
Local Name	:	Siruthutthi

1.1.2. Fabric

Desized, scoured and bleached cotton fabric was used for dyeing.

1.1.3. Mordants

(I) myrobolan: 1g of mordant in 20 ml water to 20 min at heated 60°c ±5°c. The mixture was filtered and the filtrate and the filtrate stocked for use.

(II) Therminaliabellirica: 1g of mordant in 20 ml water to 20 min at heated 60°c ± 5°c. The mixture was filtered and the filtrate and the filtrate stocked for use.

(III) Banana stem juice: The mordant were grained in to juice with the help of mixer granter. The mixture was filtered and the filtrate and the filtrate stocked for use.

(IV) Curcuma Aromatica2g of mordant in 10 ml water 60 min at heated 70°c ±5°c. The mixture was filtered and the filtrate and the filtrate stocked for use.

(V) Cow dung: Fresh cow – dung (2g) was collected from the Local Area. Water (10 ml) was added and the mixture stirred. The mixture was filtered and the filtrate and the filtrate stocked for use.

2.0. Experimental

2.1. Dye Extraction

200 gram of fresh flowers was weighed and taken in soxhlet apparatus and 500 ml of solvent (ethanol water) in the ratio 80:20 was added to it. The sox let apparatus was heated 70°c for 60 min. After extraction, the extract was filtered and used for dyeing.

2.2. Dyeing procedure

The cotton samples were dyed with dye extract keeping M:L ratio as 1:20 Dyeing was carried out at 78°c and continued for 1hour.

2.3. Mordanting

The cotton samples were treated and with different Naturalmordants by following three methods.

(i) pre-mordanting
(ii) simultaneousmordanting
(iii)post-mordanting

3.0. PRE-MORDANTING METHODS

Pre-mordanting of cotton fabrics with metallic salts

Soaked cotton fabric with or without pre-mordanting were further mordanted prior to dyeing of any one of the Natural mordants, such as Myrobolan, Cow dung, *Banana steam juice*, Curcuma Aromatica, Therminalica Belliricaat 60°C for 30 minutes with materialto-liquor ratio of 1:80. The samples treated with metal salts were dyed with the dye extract

(ii) Simultaneous mordanting

Simultaneous mordanting of cotton fabrics with metallic salts

Soaked cotton fabrics were treated with both dye extract and Natural Mordants simultaneously, of any one of the Naturalmordants, such as Myrobolan, Cow dung, *Banana steam juice*, Curcuma Aromatica, TherminalicaBellirica, at 60°C for 30 minutes with materialto- liquor ratio of 1:80.

(iii) Post-mordanting

Post-mordanting of cotton fabrics with Natural Mordants.

Soaked cotton fabrics were dyed with dye extract. The wetted out cotton samples were entered into different dye baths containing required amount of dye extract and water. After 15 min required amount of sodium chloride was added. After 30 minutes required amount of sodium carbonate was added. The dyeing was carried out for one hour at 60°C. The dyed samples were taken out, squeezed and used for treatment with Natural mordants process without washing. The dyed cotton samples were treated with different Natural mordantsof any one of the Natural mordants, such as Myrobolan, Cow dung, *Banana steam juice*, Curcuma Aromatica, Therminalica Bellirica, at 60°C for 30 minutes with material-to-liquor ratio of 1:80. In all the above three methods, after the dyeing is over, the dyed samples were repeatedly washed with water and then dried in air.

Color fastness

The dyed samples were tested according to IS standards. color fastness to washing, light, rubbing and perspiration were determined from standard test methods.

4.0. Dye Extraction

200 gram of fresh flowers was weighed and taken in soxhlet apparatus and 500 ml of solvent (ethanol water) in the ratio 80:20 was added to it.

The soxhlet apparatus was heated 70°c for 60 minutes. After extraction, the extract was filtered and used for dyeing. It was observed that, colour of the dye extract was milky yellow colour as shown in figure 2.

Hibiscus vitifolius L.

5.0. Result and Discussion

5.1. Dyeing Behavior of Dye Extract

Soxhlet Extraction Method We carried out this extraction method in order to avoid the filtration of the solvent and residue and also to obtain better efficiency of separation .in this method, the weighed quantity of feed or raw material and measured volume of solvent were taken in certain F/S ratio. The raw material was kept in thimble of soxhlet extractor and the solvent was poured in the round bottom flask and a condenser with high flow rate of water is fitted over it. The cotton fabric was dyed with Naturalmordants. It was observed that, the dye uptake was found to be good in Therminalica Bellirica at Simultaneous Mordanting method is show in figure-3.

Table : 1

Mordanting Method	Mordant Proportions	Light Fastness Grades	Washing Fastness Grades		Rubbing Fastness Grades		Perspiration Fastness			
			CC	CS	Dry	Wet	CC	CS	CC	CS
SIM mordants	Myrobolan	3	3	4	4-5	3-4	4-5	3-4	4-5	3
	Cow dung	3	3	4-5	4-5	3-4	4	4	4-5	3-4
	Banana steam juice	3	2	4-5	4-5	4	3-4	3-4	4	3
	Curcuma Aromatica	3	1-2	4-5	4	3-4	4	3-4	3-4	3
	Therminalica Bellirica	4	3-4	4-5	4-5	3-4	4-5	3-4	4-5	3

Effect of Mordanting

The dye extract was found to be suitable for cotton fabric. The cotton fabrics were dyed with Naturalmordants. It was observed that, the dye uptake was found to be good in Therminalica Bellirica at Simultaneous Mordanting method is shown in figure – 1.

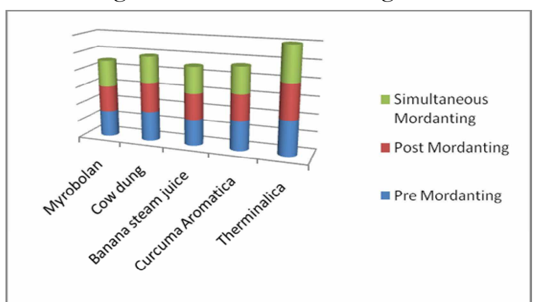


figure – 1
Light Fastness after Pre, Post and Simultaneous Mordanting

Effect of M:L ratio

The cotton samples were dyed with dye extract keeping various M:L ratio as 1:60, 1:70, 1:80 and 1:90 It was observed that the dye uptake was good in M:L ratio 1:80.

5.2. Fastness properties

The fastness properties of dyed of cotton fabrics are shown in Table3.it was observed that, dyeing with *Hibiscus vittifolius.L, Fioriavitifolia(L.) Mattei.*gave good washing, light and rubbing fastness properties.

Evaluation of color fastness Colorfastness to washing

Wash fastness of all dyed samples was measured by the ISO-105-C06: 2010 testing method. Method. Dyed samples were taken, stitched with one of the shorter side of the

adjacent bleached fabric and was put to the bath containing 0.4% ECE (B) Refedence Detergent and 0.1% of sodium perborateand 1:30 MLR ratio at 40°C for 30 minutes. Then the specimen was washed with hot water, cold water and then it was dried. Then the dried fabrics were evaluated for color change and staining using grey scale.

2.11.2. Rubbing Fastness (IS: 766-1956)

Two sample pieces of not less than 14x5 cm, one piece having the long direction parallel to the warp yarn and the other parallel to the weft yarns were cut. These two pieces were used for dry rubbing test and two other similar pieces were cut and used for wet rubbing test. A Crock meter was used as the rubbing device. The untreated fabric was used as the control.

2.11.2.1. Dry Rubbing

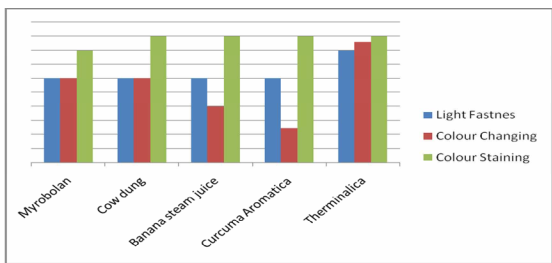
One test piece was taken and fixed to the rubbing device. A piece of the dry treated fabric was fixed in place over the end of the finger of the running device and rubbed to and fro in a straight line along a 10 cm long track on the (dry) test piece ten times in ten seconds with a downward force of 900g on the finger. The second test piece of untreated cloth was treated in a similar manner.

2.11.2.2. Wet Rubbing

One test piece (dry) was taken and fixed to the rubbing device. The test piece was soaked in distilled water and squeezed so that it contained its own weight of water. The wet piece of the test piece was placedover the end of the finger of the testing device and rubbed it to and fro in a straight line along a 10 cm long track on the dry test piece ten times in the seconds with a downward force of 900g on the finger. The piece was dried at room temperature. The second piece of the untreated fabric was treated in a similar manner. The degrees of the staining of the treated and untreated fabrics were evaluated with the help of geometric grey scales (staining) and the numerical ratings were assigned.

2.11. 3. Light Fastness (ISO 105-BO2 : 2014)

Light fastness was evaluated according to ISO 105-BO2 : 2014. Each treated and untreated fabric samples were exposed to xenon arc lamp for 20 hours. After that the change in color of the test fabric samples were evaluated with the help of geometric grey scales and the numerical ratings were assigned for both treated and untreated fabrics.



1. Very poor, 2. Poor, 3. Medium 4. Good 5. Excellent
Light Fastness and Simultaneous Mordanting
Figure – 3

2.11.4. Acidic and Alkaline Perspiration (ISO: E04: 2013)

The acidic test liquor was prepared by dissolving 2.65g of sodium chloride and 0.75g of urea per liter and the pH was adjusted to 5.6 with the addition of acetic acid. The alkaline test liquor was prepared by dissolving 3g of sodium chloride per liter and the pH was adjusted to 7.2 with the addition of sodium bicarbonate. One of the composite specimen was wetted thoroughly in the acidic test liquor using liquor to specimen ratio of 50:1 (mg) and allowed to retain in the liquor for 30 minutes at room temperature. Care was taken specially while wetting the specimen to see that it was uniformly saturated. The liquor was poured off and the specimen was placed in between two glass plates under a force of 4.5 kg. The glass plates were kept with the specimens in the perspirometer and placed in the hot air oven for 4 hours at 370 C. At the end of this period, the specimen was removed and the test piece was separated from the two pieces of the untreated fabric and dried in air at a temperature not exceeding 600 C. The second composite specimen was also treated similarly using alkaline test liquor. The change in color of the test pieces (treated in both acidic and alkaline

broth) and the degree of staining of the corresponding two pieces of untreated cloth were evaluated with the help of geometric grey and the numerical ratings were assigned for both treated and untreated fabrics.

Mordants	Colour obtained
Myrobolan	
Cowdung	
Banana Stem Juice	
Curcuma aromatica	
Therminaila Bellirica	

Fig-3: Colour produced on Cotton by different mordants in Simultaneous Mordanting

6.0. Conclusion

The natural dying solutions were obtained by extraction from*Hibicusvittifolius.L, Fioriavitifolia(L.) Mattei*and used for dyeing cotton fabrics. Dyeability and fastness properties of the dyed sample were studied. The natural extracts with cotton fabric had more affinity towards and more stability of colorant to light, rubbing, washing and perspiration. The fabric dyed with the natural colorant from Therminalica Bellirica Excellent. Hence, this dye will not cause any skin problems to the wearer and also will not pollute the environment.

References

1. *Lokesh P* and Kumara Swamy M Pelagia* Research Library Extraction of natural dyes from *Spathodeacampanulata*and its application on silk fabrics and cotton. Der Chemica Sinica, 2013, 4(1): 111-115
2. *Wan Yunus Wan Ahmad, Razis Rahim*, Mohd Rozi Ahmad, Muhammad Ismail Abdul Kadir, MohdIqba lMisnon* The Application of *Gluta Aptera* Wood (Rengas) as Natural Dye on Silk and Cotton Fabrics. Volume 1, Issue 4: 545-551.
3. *1R.Rajendran and 2 B.ThamaraiSelvi* Natural Dyeing of Cotton Fabrics with Pigment Extracted from Roseomonas Fauriae.2014 Volume 4, Issue1:54-59
4. *ShyamVir Singh1* and M. C. Purohit2*Dyeing of Wool Fabrics with Extracted Eco-Friendly Natural Dyes from *RhusParviflora*(Tung) Using Chemical Mordants: Comparison of Fastness Properties and Colour Strength with Some Mordants. Chemical Science Transactions 2014, 3(3), 1124-1130.
5. *Kannadasan T.1, Gnanaprakasam A.1, Harikrishnan R.3*, Nganyei konyak3, RajkumarA.3* Journal of Advanced Scientific Research.ISSN 0976-9595.
6. *Pravinkumar. D. Patil1, C.R. Rao2, A.I. Wasif3, J.R. Nagla4* Taguchi optimization for efficient extraction of a natural dye from bougainvillea glabra bracts. International journal of research in Engineering and Technology eISSN: 2319-1163|Pissn:2321-7308.
7. *K. Anitha and S.N. Prasad,* 2007. Developing multiple natural dyes fro flower parts of Gulmohur, *Current Science*, Vol.92, No.12, 25.
8. *H.T.Loknande, Vishnu A and Dorugade*, 1999, Dyeing Nylon with Natrual Dyes, *American Dyestuff Report.*
9. *Meenu Srivatstava and Preeti Udawat, 2008*, Dyeing of silk with natural dye extracted from polygonumamplexicaule, *Man-made textiles in India.*
10. *Kulkarni. S.S, Gokhale. A.V, Bodake. U.M and Pathade. G.R, 2011*, Cotton dyeing with Natural Dye Extracted from Pomegranate Peel, *Universal Journal of Environmental Research and Technology*, Vol. 1(2): 135-139.
11. *M. Kumaresan, P.N. Palnisamy and P.E. Kumar, 2011*, Application of Ecofriendly Natural Dye Obtained from flower of Spathodea Campanulata on Silk, *European Journal of Scientific Research,*Vol.52, No.3, pp.306-312.
12. Saravanan P.[1], Chandramohan G.[2], MariajancyraniJ.[2] and Shanmugasundaram P.[3] Extraction and application of Eco-Friendly Natural dye obtained from Leaves of Acalyphaindica Linn on Cotton Fabric, International research journal of environment science. *Vol 2 (12),1 – 5, December (2013).*